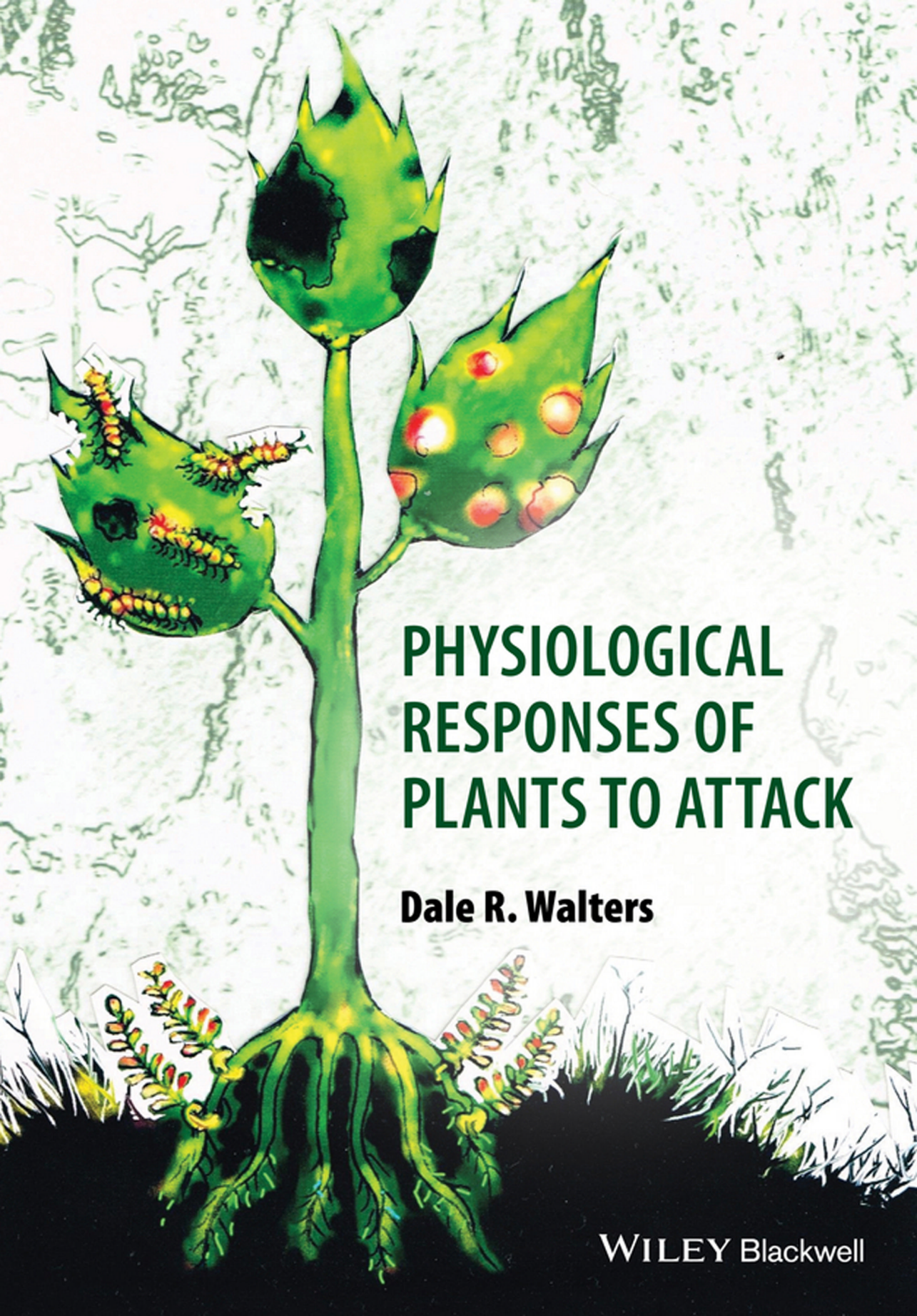




PHYSIOLOGICAL RESPONSES OF PLANTS TO ATTACK

Dale R. Walters

WILEY Blackwell

VISIT...

LANZAROTE
Caliente.COM

Physiological Responses of Plants to Attack

Physiological Responses of Plants to Attack

Dale R. Walters

Crop & Soil Systems Research Group

SRUC

Edinburgh, UK

WILEY Blackwell

This edition first published 2015 © 2015 by Dale R. Walters

Registered office: John Wiley & Sons, Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

Editorial offices: 9600 Garsington Road, Oxford, OX4 2DQ, UK

The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK

111 River Street, Hoboken, NJ 07030-5774, USA

For details of our global editorial offices, for customer services and for information about how to apply for permission to reuse the copyright material in this book please see our website at www.wiley.com/wiley-blackwell.

The right of the author to be identified as the author of this work has been asserted in accordance with the UK Copyright, Designs and Patents Act 1988.

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, except as permitted by the UK Copyright, Designs and Patents Act 1988, without the prior permission of the publisher.

Designations used by companies to distinguish their products are often claimed as trademarks. All brand names and product names used in this book are trade names, service marks, trademarks or registered trademarks of their respective owners. The publisher is not associated with any product or vendor mentioned in this book.

Limit of Liability/Disclaimer of Warranty: While the publisher and author(s) have used their best efforts in preparing this book, they make no representations or warranties with respect to the accuracy or completeness of the contents of this book and specifically disclaim any implied warranties of merchantability or fitness for a particular purpose. It is sold on the understanding that the publisher is not engaged in rendering professional services and neither the publisher nor the author shall be liable for damages arising herefrom. If professional advice or other expert assistance is required, the services of a competent professional should be sought.

Library of Congress Cataloging-in-Publication Data

Walters, Dale, author.

Physiological responses of plants to attack / Dale R. Walters.

pages cm

Includes bibliographical references and index.

ISBN 978-1-4443-3329-9 (pbk.)

1. Plant-pathogen relationships. 2. Plant physiology. I. Title.

SB732.7.W35 2015

632—dc23

2014041920

A catalogue record for this book is available from the British Library.

Wiley also publishes its books in a variety of electronic formats. Some content that appears in print may not be available in electronic books.

Cover image by Archie Graham

Set in 10/12pt Times by Laserwords Private Limited, Chennai, India

To Beverley

Contents

<i>Preface</i>	xi
1 The Interaction Between a Plant and Its Attacker	1
1.1 Introduction	1
1.2 Different types of attacker	1
1.3 Symptoms exhibited by plants following attack	20
1.4 Conclusions	21
Recommended reading	21
References	22
2 Growth, Development and Yield of Infected and Infested Plants and Crops	24
2.1 Introduction	24
2.2 Effects of pathogens on growth, development and yield	24
2.3 Effects of nematodes on growth, development and yield	29
2.4 Effects of herbivores on growth, development and yield	30
2.5 Effects of parasitic plants on growth, development and yield	36
2.6 Conclusions	37
Recommended reading	38
References	38
3 Photosynthesis in Attacked Plants and Crops	41
3.1 Introduction	41
3.2 Photosynthesis in diseased plants	41
3.3 Photosynthesis in plants infected with nematodes	61
3.4 Photosynthesis in plants infested with insects	65
3.5 Photosynthesis in plants infected with parasitic plants	73
3.6 The caring robber? hardly!	80
3.7 Conclusions	81
Recommended reading	81
References	81

4	Respiration in Plants Interacting with Pathogens, Pests and Parasitic Plants	88
4.1	Introduction	88
4.2	Effects of attack on respiration	90
4.3	Photorespiration in attacked plants	105
4.4	Conclusion	108
	Recommended reading	109
	References	109
5	Effects on Carbohydrate Partitioning and Metabolism	114
5.1	Introduction	114
5.2	Carbohydrate partitioning and metabolism in plants infected by pathogens	114
5.3	Carbohydrate metabolism and partitioning in plant–insect herbivore interactions	122
5.4	Carbohydrate metabolism and partitioning in interactions between plants and parasitic angiosperms	124
5.5	Conclusions	125
	Recommended reading	126
	References	127
6	Water Relations of Plants Attacked by Pathogens, Insect Herbivores and Parasitic Plants	130
6.1	Introduction	130
6.2	Effects of pathogens on plant water relations	130
6.3	Effects of nematodes on plant water relations	139
6.4	Water relations in plants infested with insect herbivores	140
6.5	Effects of parasitic angiosperms	145
6.6	Conclusions	148
	Recommended reading	149
	References	149
7	Mineral Nutrition in Attacked Plants	153
7.1	Introduction	153
7.2	Mineral nutrition in plant–pathogen interactions	156
7.3	Mineral nutrition in plant–nematode interactions	164
7.4	Mineral nutrition in plant–insect interactions	165
7.5	Mineral nutrition in interactions between plants and parasitic angiosperms	170
7.6	Conclusions	175
	Recommended reading	176
	References	176

8	Hormonal Changes in Plants Under Attack	181
8.1	Introduction	181
8.2	Hormonal changes in plants responding to pathogens	181
8.3	Hormonal changes in plants responding to insect attack	198
8.4	Hormonal changes in plants infected with parasitic plants	201
8.5	Conclusions	205
	Recommended reading	207
	References	207
9	Bringing It Together: Physiology and Metabolism of the Attacked Plant	215
9.1	Introduction	215
9.2	Metabolic reprogramming in plant–pathogen interactions	215
9.3	Metabolic reprogramming in interactions between plant and parasitic nematodes	220
9.4	Metabolic reprogramming in plant–insect interactions	221
9.5	Metabolic reprogramming in interactions between plants and parasitic angiosperms	222
9.6	Metabolic reprogramming – is the plant just a bystander in compatible interactions?	222
9.7	Plant responses to attack – a look to the future	222
	Recommended reading	223
	References	223
	<i>Index</i>	225

Preface

The continued existence of plants is remarkable given the huge range of organisms that uses them as a source of nourishment. The fact that plants survive in the face of continual onslaught from attackers is testimony to their defensive abilities and their ability to cope with damage inflicted during attacks. Understanding the changes that occur in plants under attack is important in attempts to produce crops better able to withstand the ravages of pathogens and pests. Feeding an ever-increasing human population requires not only efficient crop production, but also the ability to protect crops, allowing them to realise their yield potential. In the study of crop protection, plant defence has attracted most attention from researchers. However, it is becoming increasingly clear that understanding the metabolism and physiology of interactions between plants and their attackers is important, not least because of the connections between plant defence and primary metabolism. The interaction between a plant and an attacker is dynamic, and, for example, in an incompatible interaction, host defence is financed by primary metabolism, and often, effective resistance is associated with a cost in terms of plant growth. In compatible interactions, despite the fact that attackers are able to manipulate host metabolism for their own benefit, the host plant is still able to alter metabolic processes to make life difficult for the invader.

We are beginning to understand interactions of plants with the biotic environment at a level of detail that was difficult to imagine when I was an undergraduate student at Wye College in the mid-1970s. My interest in what was then called ‘physiological plant pathology’ started at Wye, but it was my move to Lancaster for postgraduate work that cemented my interest in plant disease physiology. I was very fortunate to be supervised for my PhD by Peter Ayres whose gentle approach to supervision and enthusiasm for physiological plant pathology made my time at Lancaster very happy. Over the years, I have been very fortunate to be able to discuss ideas with various colleagues, especially Nigel Paul, Ian Bingham and Adrian Newton. I am most grateful to Nigel Balmforth, who has always been supportive of my ideas for books and has shown considerable patience when I’ve asked for deadline extensions. Finally, I owe a huge debt of gratitude to Beverley for not only encouraging me in my book-writing activities, but also putting up with my grumpiness when the writing is not going well.

I have taught modules on the physiological responses of plants to pathogens since 1982, and over the years, this has developed to include physiological responses to pests and parasitic plants. It appears logical to me to study plant responses to different attackers in the same module, and in the absence of a single text adopting this approach, I decided to write one. It took me longer than expected, and there were times I thought I’d taken on too big a task, but the more I delved into the literature, the more fascinated I became. I hope this fascination comes through in the following chapters.

Dale R. Walters
SRUC
Edinburgh, UK

1 The Interaction Between a Plant and Its Attacker

1.1 INTRODUCTION

Plants are the only higher organisms on the planet capable of converting energy from the Sun into chemical forms of energy that can be stored or used (Agrios, 2005). Not surprisingly therefore, plants are a source of food for a great many organisms. Indeed, directly or indirectly, plants are a source of nourishment for all humans and animals. Although plants have evolved a bewildering array of defences with which to ward off attack (Walters, 2011), many plants succumb to attack and suffer damage and disease as a result. This, in turn, can affect the growth and reproductive output of the plant, which can exert a significant effect on competitive ability and survival. In terms of crop production, damage and disease can affect the yield and quality of produce, with economic consequences to the farmer or grower. In this book, we examine the mechanisms responsible for the changes in plant growth, development and yield following attack by various organisms. Such knowledge is important because it can be useful in our attempts to protect crops from attack, as well as helping them to cope with the consequences of attack.

Plants that are attacked are likely to show visible signs of the encounter and the resulting after effects. Symptoms can be useful, not only in identifying an affected plant, but also in hinting at the cause of the problem and even the nature of the attacker. We look at symptoms in some detail later in this chapter, but let us turn our attention first to the attackers, because the nature of the attacker and the way it obtains food from the plant can exert a profound influence on the way the plant responds and the symptoms we observe.

1.2 DIFFERENT TYPES OF ATTACKER

The range of organisms that use plants as a source of food includes microorganisms, nematodes, insects, vertebrates and other plants. The major microorganisms attacking plants are fungi, bacteria and viruses, some of which can have devastating effects on plants. Herbivory by insects, invertebrates and vertebrates can also lead to considerable damage and plant death,

while plants are not safe even from other plants, as some have evolved the parasitic habit, with serious economic consequences.

1.2.1 Microorganisms

Microorganisms can obtain food from plants by a number of routes. Some live on dead material, decomposing plant tissues and releasing nutrients that would otherwise remain unavailable to other organisms. These microbes are known as saprotrophs, and they subsist entirely on organic debris. Other microbes have developed the ability to infect plants, living as parasites, taking nourishment from the living plant but giving nothing back in return. If such parasitic microbes, as a result of their association with the host plant, also lead to disruptions in normal functioning of the plant, they are defined as pathogens, and the plant is said to be diseased. Some pathogens infect a living plant, but then kill all or part of their host rapidly, and survive on the dead plant tissues. These are known as necrotrophs, while those pathogens that infect the plant and then coexist with it for an extended period, causing little damage, are known as biotrophs. Although it might appear that biotrophy and necrotrophy represent absolute categories, they are actually at opposite ends of a continuum (Walters *et al.*, 2008; Newton *et al.*, 2010). At one end of the continuum are pathogens that require living host cells to survive, such as viruses and biotrophic fungi, for example powdery mildews and rusts, while at the other end are the necrotrophic pathogens such as damping-off fungi and soft rot bacteria. As one moves from one end of this continuum to the other, one encounters pathogens with intermediate characteristics. Some of these pathogens possess an initial biotrophic phase in their life cycle, during which they cause little, if any, damage to plant cells and tissues, but then move into a necrotrophic phase, where plant cells and tissues are killed. These pathogens have been termed hemibiotrophs and include the late blight pathogen *Phytophthora infestans* and the pathogenic bacterium *Pseudomonas syringae*. The triggers responsible for the transition between the biotrophic and necrotrophic phases in these pathogens are not known (Newton *et al.*, 2010).

1.2.1.1 Fungi

The vegetative phase of fungi may be quite limited, occurring, for example, as single cells (yeasts) or may be more extensive. For most plant pathogenic fungi, vegetative growth is as filamentous hyphae, which grow by extension at the tips. These hyphae can form a network known as a mycelium, while the interconnected network of hyphae derived from one fungal propagule is known as a colony. The lifespan of the colony and its functional relationship with the growing hyphal tips vary depending on the fungus. Thus, in pathogenic fungi belonging to the genus *Pythium*, as hyphal tips grow and extend, the older parts of the colony die. In these fungi, sporulation occurs at the advancing edge of the colony. Although the hyphal lifespan in fungi such as *Pythium* is short, in other fungi, hyphae live for considerably longer. Good examples are the runner hyphae produced by the take-all fungus *Gaeumannomyces graminis* and rhizomorphs produced by the tree pathogen *Armillaria mellea*. These hyphae grow on plant surfaces or away from the host plant, exposing them to harsh environments. As a result, they possess thick, dark-coloured walls, enabling them to withstand desiccation and the vagaries of the aerial or soil environments. Indeed, the rhizomorphs produced by *A. mellea* are large, elaborate structures, with thick, pigmented walls. Runner hyphae and rhizomorphs allow the fungus to grow from one host plant to another, with nutrients transported from the

older, established parts of the colony, to the expeditionary hyphae seeking new sources of nourishment. In contrast, colonies in biotrophic fungal pathogens such as rusts and powdery mildews remain functional for long periods, with nutrients transported from hyphae at the outer edges of the colony to the colony centre. In this case, the older, central portion of the colony remains functional and is associated with important developmental processes such as sporulation.

1.2.1.2 *Bacteria*

Although bacteria are important as pathogens of animals, including man, relatively few are known to be plant pathogens. Bacteria are prokaryotic. In other words, they possess no nuclear membrane or mitotic apparatus, and additionally, mitochondria and a visible endoplasmic reticulum are lacking. Most bacteria are unicellular, although some occur in groups or chains of cells. Bacterial cells are small (5–10 µm), and some are rod shaped (bacilli) or spherical (cocci), while others have unusual shapes. All plant pathogenic bacteria are rod shaped, and many possess flagella, making them motile and capable of moving along nutrient gradients.

Within the plant, bacterial cells can spread throughout an organ, as is the case with soft rot bacteria in potato tubers, or can spread widely in the plant, as with vascular wilt bacteria, which can be spread throughout the plant in the xylem.

1.2.1.3 *Viruses*

Most plant viruses consist of a single strand of RNA surrounded by a protein sheath (the capsid), although a few consist of double-stranded RNA or of DNA. In fact, five classes of plant virus have been described on the basis of whether the nucleic acid is RNA or DNA, whether it is single or double stranded and whether the strand is of the same (+) or opposite (–) polarity to messenger RNA (Table 1.1). Most plant viruses described to date belong to Class IV, consisting of single-stranded RNA. Inside the plant cell, once this single strand of RNA is freed from its protein coat, it can act as messenger RNA in the synthesis of new virus particles. Examples of plant viruses belonging to Class IV include tobacco mosaic virus (TMV) and cucumber mosaic virus (CMV). Viral parasitism is unique, because viruses act as ‘molecular pirates’, hijacking the synthetic machinery of the plant to make more virus particles (Lucas, 1998).

Class VII in Table 1.1 contains viroids. These differ from viruses in the size of their RNA genome and the fact that they lack a protein coat. A viroid consists of a single stranded but covalently closed RNA molecule, ranging in size from 246 to 401 nucleotides. They do not encode any pathogen-specific peptides, and they replicate autonomously. Viroids can be classified into two major families, the *Pospiviroidae* (e.g. the potato spindle tuber viroid RNA) and the *Avsunviroidae* (e.g. avocado sunblotch viroid) (Tabler & Tsagris, 2004).

1.2.1.4 *Phytoplasmas*

Phytoplasmas are wall-less bacteria that inhabit the phloem and are known to cause disease in more than a thousand plant species. They are transmitted by phloem-feeding insects, mainly leafhoppers, planthoppers and psyllids. In 2004, phytoplasmas, known previously as mycoplasma-like organisms, were assigned to the novel provisional genus *Candidatus* Phytoplasma (Firrao *et al.*, 2005). They represent a monophyletic group within the class

Table 1.1 The Baltimore system for virus classification, based on the type of nucleic acid present (RNA or DNA), whether it is double (ds) or single stranded (ss) and whether the strand is of the same (+) or opposite (−) polarity to messenger RNA.

	Genome	Examples of plant viruses
Class I	ds(±)DNA	Cauliflower mosaic virus (CaMV)
Class II	ss(+)DNA	Gemini viruses, e.g. African cassava mosaic virus (ACMV)
Class III	ds(±)RNA	Wound tumour virus (WTV)
Class IV	ss(±)RNA	Tobacco mosaic virus (TMV)
Class V	ss(−)RNA	Rhabdoviruses, e.g. lettuce necrotic yellows virus (LNyV)
Class VI	ss(+)RNA transcribed to DNA for replication	No plant-infecting examples known
Class VII	ssRNA does not contain structural genes and has no protein coat	Viroids, e.g. potato spindle tuber viroid

Source: Adapted from Lucas (1998). Reproduced with permission of John Wiley & Sons.

Mollicutes (trivial name, mycoplasmas) and are thought to have evolved from gram-positive bacteria (Maniloff, 2002). In contrast to most mycoplasmas, phytoplasmas cannot be grown in culture and, as a consequence, are poorly characterised on a physiological and biochemical basis. Diseases caused by phytoplasmas include chrysanthemum yellows, clover phyllody, soybean phyllody, elm witches' broom and pear decline.

1.2.1.5 The host–pathogen interface

The site of contact between the host cell and the pathogen is known as the host–pathogen interface, and five types of interface can be distinguished (Table 1.2). Pathogens that grow intercellularly have no intimate contact with living host cells but rather grow between cell walls and in the spaces between cells. This apoplastic space contains various soluble nutrients, such as sugars and amino acids, which can be taken up by pathogens. Some intercellular pathogens are necrotrophic, secreting hydrolytic enzymes or toxins, which kill host cells in advance of invasion, making any interface between host and pathogen short-lived. A rather different and in many cases, longer-lasting interface, is observed with intracellular pathogens. In the interaction between the club root pathogen *Plasmodiophora brassicae* and a brassica host, the interface consists of the membrane of the pathogen cell or plasmodium, surrounded by another membrane that is assumed to be of host origin. Another pathogen attacking roots of brassicas, *Olpidium brassicae*, has an even more intimate interface with the host cell. In this case, the fungal cell is in direct contact with the cytoplasm of the host, as it is not surrounded by a host-derived membrane. The ultimate in terms of an intracellular interface must surely lie with viruses and viroids, because during virus replication, the host–pathogen interface is between a nucleic acid molecule and the nucleic acid synthetic machinery of the host cell.

Many biotrophic and hemibiotrophic fungal pathogens have a long-lasting intracellular relationship where host cells remain viable for a prolonged period. In many cases, the host–pathogen interface involves the formation of specialised structures known as haustoria, which represent the hallmark of obligate biotrophs such as powdery mildews, rusts and

Table 1.2 Modes of pathogen growth within host tissues and host–pathogen interfaces.

Type	Pathogen	Host
Subcuticular	<i>Rhynchosporium</i>	Barley
	<i>Venturia</i>	Apple
Intercellular	<i>Cladosporium</i>	Tomato
	<i>fulvum</i>	Bean
	<i>Sclerotinia</i>	Pear
	<i>Monilinia</i>	Various
	Most bacteria	
Vascular	<i>Fusarium</i>	Various
	<i>Verticillium</i>	Various
	<i>Ophiostoma</i>	Elm
	Some bacteria, phytoplasmas	
Haustorial		
Epiphytic with haustoria	Powdery mildews	Various
Intercellular with haustoria	Rust fungi	Various
	<i>Hyaloperonospora</i> <i>parasitica</i>	Brassicas
Intracellular vesicle, with intercellular hyphae and haustoria	<i>Bremia</i>	Lettuce
	<i>Phytophthora</i>	Potato
Intracellular		
Vesicle and intracellular hyphae	<i>Colletotrichum</i>	Bean
	<i>Pyrenophora</i>	Wheat
Wholly intracellular	<i>Plasmidiophora</i>	Cruciferae
	<i>Polymyxa</i>	Cereals, beet
	Viruses	Various

Source: Adapted from Lucas (1998). Reproduced with permission of John Wiley & Sons.

oomycetes. They develop as side branches from intercellular, intracellular and epicuticular hyphae and terminate inside the host cell (Fig. 1.1; Voegelé & Mendgen, 2003; O'Connell & Panstruga, 2006). Some hemibiotrophs, such as species of *Colletotrichum* and *Magnaporthe*, and obligate biotrophs such as the monokaryotic rust *Uromyces vignae*, produce filamentous intracellular hyphae, which, rather than terminating in the first penetrated host cell, penetrate from cell to cell, thereby colonising a small number of host cells (e.g. Wharton *et al.*, 2001). Once these haustoria and intracellular hyphae (IH) have breached the host cell wall, they develop inside the cell but never penetrate the host plasma membrane. With haustoria, this gives rise to an interface comprising the plasma membrane and cell wall of the biotrophic pathogen, a plant-derived interfacial membrane (known as the extrahaustorial membrane, EHM), and an interfacial matrix layer (the extrahaustorial matrix, EHMA) (Fig. 1.2). In most haustoria, a discrete, electron-dense ring is visible in the fungal cell wall in the neck region (Fig. 1.2). This neck band is not observed in haustoria formed by oomycete pathogens. Haustoria are diverse in morphology, ranging from small, club-shaped extensions, to larger, branched structures (Fig. 1.2).

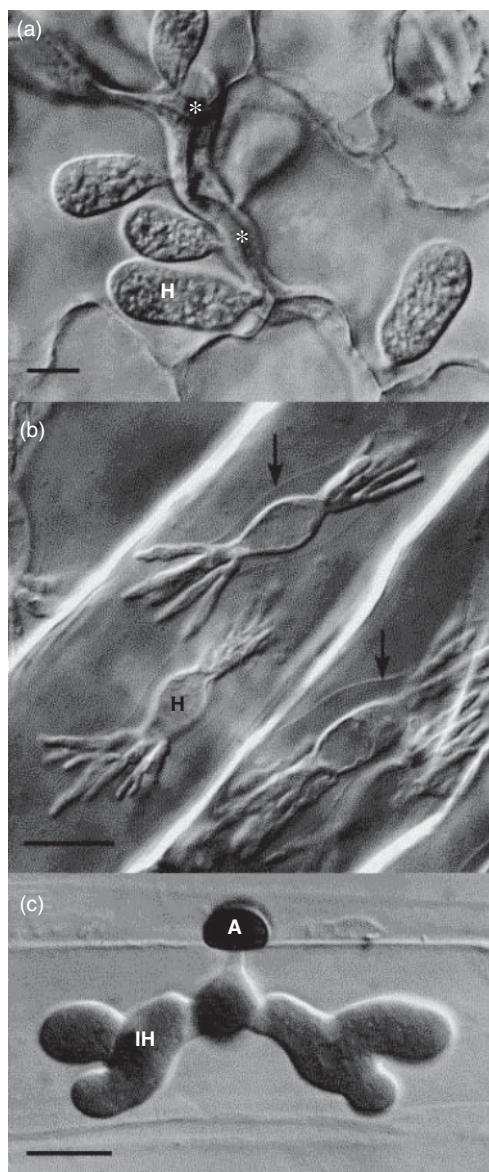


Fig. 1.1 Light micrographs illustrating the infection structures of some intracellular biotrophs. (a) Haustoria (H) developing from intercellular hyphae (*) of the obligately biotrophic oomycete *Hyaloperonospora parasitica* inside epidermal cells of *Brassica oleracea* (b) Haustoria (H) of the obligately biotrophic powdery mildew fungus *Blumeria graminis* f.sp. *avenae* developing inside epidermal cells of *Avena fatua*. Arrows indicate the EHM. (c) Intracellular hyphae (IH) of the hemibiotrophic crucifer anthracnose fungus *Colletotrichum higginsianum* have developed from a melanized appressorium (A) and penetrated into an epidermal cell of *Arabidopsis thaliana*. Bars, 10 μ m. Image (a) was provided by Raffaella Carzaniga, Rothamsted Research, Hertfordshire, UK. Image (b) was provided by George Barron from the MycoAlbum CD-ROM, University of Guelph, Guelph, Ontario, Canada. Image (c) was provided by Richard O'Connell. O'Connell and Panstruga (2006). Reproduced with permission from John Wiley & Sons.

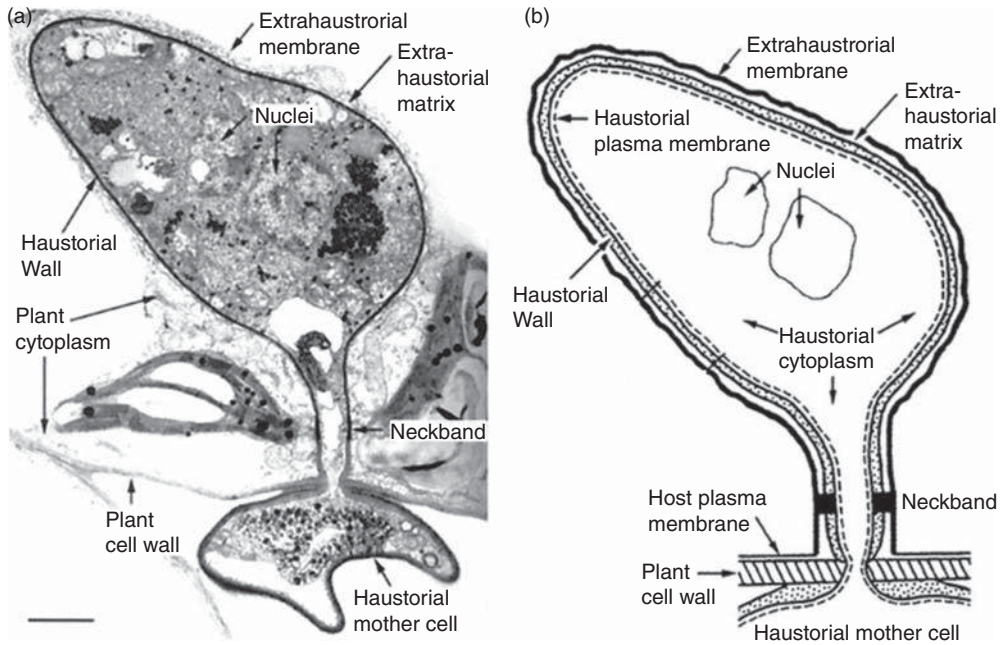


Fig. 1.2 (a) Transmission electron micrograph of a flax rust haustorium. (Bar, 1 μm .) (b) Drawing showing key features of the fungal haustorium. To move from host cell to fungus, nutrients must traverse the extrahaustorial membrane, the extrahaustorial matrix, the haustorial wall and the haustorial plasma membrane. A neckband seals the extrahaustorial matrix from the plant cell wall region so that the matrix becomes a unique, isolated, apoplast-like compartment. The haustorium connects to intercellular fungal hyphae by way of a haustorial mother cell. Coffey *et al.* (1972). Reproduced with permission from Canadian Science Publishing or its licensors.

The much branched structure of haustoria provides a large surface area and, taken together with their location, frequently close to chloroplasts, suggests a role in nutrient uptake. Thus, ATPase, an enzyme involved in active solute transport, was detected in the host membrane and in the fungal plasma membrane inside the haustorium but not in the EHM. This suggested that host and fungal protoplasts import solutes actively, whereas the membrane enclosing the haustorium, with reduced control of solute transport, leaks nutrients into the extrahaustorial matrix, from where they could be taken up by the fungus. In this model, the neck band of impermeable material would prevent solutes diffusing along the haustorial wall in the neck region. Thus, the haustorial wall and the extrahaustorial matrix represent a sealed compartment, where any nutrients crossing the EHM could only enter the pathogen by active transport across the plasma membrane of the haustorium. Later work using molecular tools showed that a gene encoding a hexose transporter (*HXT1*) is highly expressed in haustoria of the rust *Uromyces fabae*. The gene is localised exclusively in the haustorial plasma membrane (HPM), where it is likely to mediate the uptake of the hexoses glucose and fructose from the extrahaustorial matrix (Fig. 1.3; Voegelé *et al.*, 2001). It would appear that the hexoses derive from the cleavage of sucrose by invertases, because an invertase (*Uf-INV1*) was found to be highly expressed in *U. fabae* haustoria, and moreover, the enzyme protein was secreted into

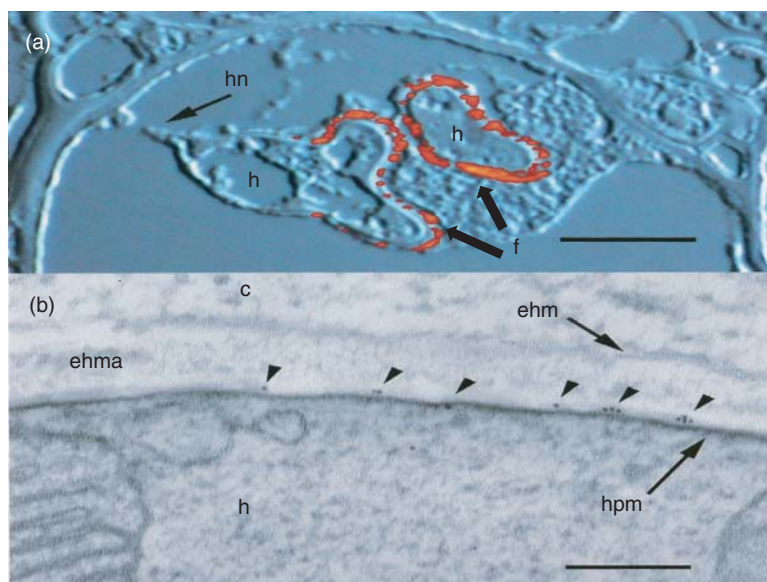
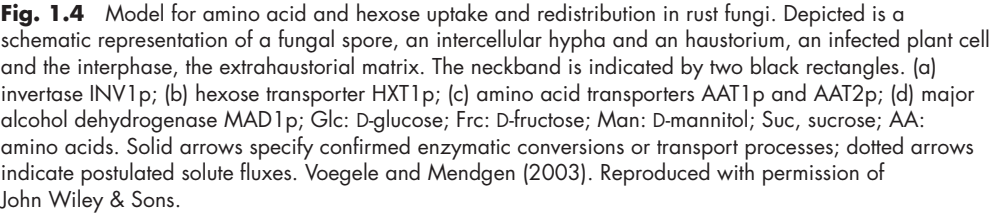


Fig. 1.3 Localization of HXT1p in the periphery of fully developed haustoria and along the HPM. (a) Superimposed Nomarski differential interference contrast and fluorescence images depicting two haustoria. Labeling of HXT1p with S651p resulted only in fluorescence signals in the periphery of the distal parts of the haustorium (f, fluorescence); proximal parts and haustorial neck are not labeled. h, haustorium; hn, haustorial neck. (Bar, 5 mm.) (b) Electron micrograph depicting considerable gold labeling along the HPM only (small arrows), but no labeling over the h, the EHMA, the EHM, or the plant cytoplasm (c). (Bar, 0.1 mm.). Voegelé *et al.* (2001). Reproduced with permission from PNAS.

the extrahaustorial matrix. Additional glucose and fructose might also be generated at the host–pathogen interface by a host cell-wall-associated invertase (*CWINV2*) (Voegelé *et al.*, 2006). Also highly expressed in *U. fabae* haustoria, as well as in intercellular hyphae, are three genes encoding amino acid transporters, suggesting that amino acids can be taken up not only by haustoria, but also by intercellular hyphae (Struck *et al.*, 2002). Interestingly, the hexose transporter protein HXT1p and the amino acid transporter protein AAT2p were localised in the apices of intracellular hyphae formed during the monokaryotic phase of *U. fabae*. This finding suggests that intracellular hyphae function as feeding structures in this fungus. Perhaps, this should be surprising, as detailed studies on colonies of the rust *Puccinia hordei* on barley estimated that haustoria accounted for less than 20% of colony surface area, while most contact between the host and the rust was between intercellular hyphae and host cell walls (Kneale & Farrar, 1985). The picture that has emerged, especially from studies on *U. fabae*, suggests that rust fungi might use two strategies for nutrient uptake from the host: uptake of amino acids via haustoria and intercellular hyphae and carbohydrate uptake by haustoria (Fig. 1.4; Voegelé & Mendgen, 2003). It is not yet known whether intracellular hyphae in hemibiotrophic fungi play any role in nutrient uptake. See Box 1.1 for more on sugar uptake by fungal pathogens.

Irrespective of the physical nature of the host–pathogen interface, it is now clear that the early stages of the host–pathogen interaction are associated with a pathogen-induced reprogramming of host metabolism. This is crucial to the establishment of a nutritional relationship



with the host, and to pathogen development, and is dealt with in Chapter 9. In an attempt to uncover mechanisms associated with the ability of a powdery mildew to satisfy its demand for host nutrients while limiting host defences, Chandran *et al.* (2010) used laser microdissection of *Arabidopsis* cells at the powdery mildew infection site. They found evidence for induced host endoreduplication, a process that increases gene copy number and could enhance the metabolic capacity of host cells at the infection site. In support of this role, they found elevated expression of genes required to increase metabolic capacity (such as genes involved in transcription, translation and energy generation), as well as genes encoding, for example, nutrient transporters. This strategy of using localised endoreduplication to meet enhanced metabolic demands has also been found in plant–nematode interactions (see Section 1.2.2).

After infection, colonisation of the host plant can be restricted to the particular tissue or organ (localised) or can be extensive, with the pathogen spreading widely within the plant (systemic).

Some pathogens colonise specific plant tissues, such as vascular wilt pathogens, which grow in the host xylem, while less specialised necrotrophic pathogens can spread indiscriminately through plant organs. The way a pathogen colonises its host can influence the type of symptoms observed and the physiological effects on the plant. However, the extent to which the pathogen colonises the host and the eventual severity of disease are not always correlated. Thus, a pathogen localised to a particular tissue, such as the xylem, can disrupt water transport, with knock-on consequences for other physiological processes, thereby exerting profound effects on the plant. In contrast, some virus infections become systemic, although the host exhibits no symptoms.

Box 1.1 Stealing sweets: sugar uptake from the host by plant pathogenic fungi

In higher plants, the main long-distance and storage form of assimilated carbon is sucrose. Indeed, sucrose concentrations in the low millimolar range have been measured in the apoplast of several plants (Nadwodnik & Lohaus, 2008). However, transport proteins identified to date from plant pathogenic and symbiotic fungi are specific for monosaccharides (e.g. Voegelé *et al.*, 2001; Polidori *et al.*, 2007). It has been suggested that host sucrose is hydrolysed extracellularly by plant and/or fungal cell wall invertases, yielding glucose and fructose for fungal uptake (Scholes *et al.*, 1994; Tang *et al.*, 1996). But herein lies a problem. It would appear that plants have evolved mechanisms to sense changes in apoplastic glucose concentrations and to respond by activating defence responses (e.g. Ehness *et al.*, 1997; Kocal *et al.*, 2008). In addition, accumulation of hexoses could lead to reductions in photosynthetic rates (Roitsch *et al.*, 2003; Rolland *et al.*, 2006), thereby reducing carbon availability to the pathogen. The evolution of feeding strategies based on sucrose uptake, avoiding the need to hydrolyse it to glucose and fructose, could therefore be highly beneficial to pathogenic fungi. Interestingly, such a strategy has been suggested for the biotrophic fungal pathogen, *Ustilago maydis*. Thus, Wahl *et al.* (2010) identified and characterised a novel sucrose transporter (Srt1) from *U. maydis*, with an affinity for sucrose that was not only very high, but also greater than the sucrose affinity of equivalent plant transporters. The possession of Srt1 would enable *U. maydis* to compete efficiently and successfully for sucrose with host cells (Fig. 1A). Moreover, it would also out-compete the invertase (INV)-dependent plant monosaccharide transporter proteins (STP), because despite being high affinity transporters, the plant extracellular invertases, which supply them with hexoses, have a low affinity for sucrose. Wahl *et al.* (2010) also found that the *srt 1* gene was expressed exclusively during infection, and importantly, its deletion greatly reduced fungal virulence.

Soon after uptake by the fungus, the host sugars are converted into fungal sugars, including the polyol, mannitol. Indeed, mannitol concentrations have been shown to increase in leaves infected with biotrophs, hemibiotrophs and necrotrophs (Voegelé *et al.*, 2005; Dulermo *et al.*, 2009; Parker *et al.*, 2009). Since mannitol is membrane impermeable, conversion of host sugars to mannitol might maintain a gradient for continued uptake and sequestration of host sugars (Lewis & Smith, 1967).

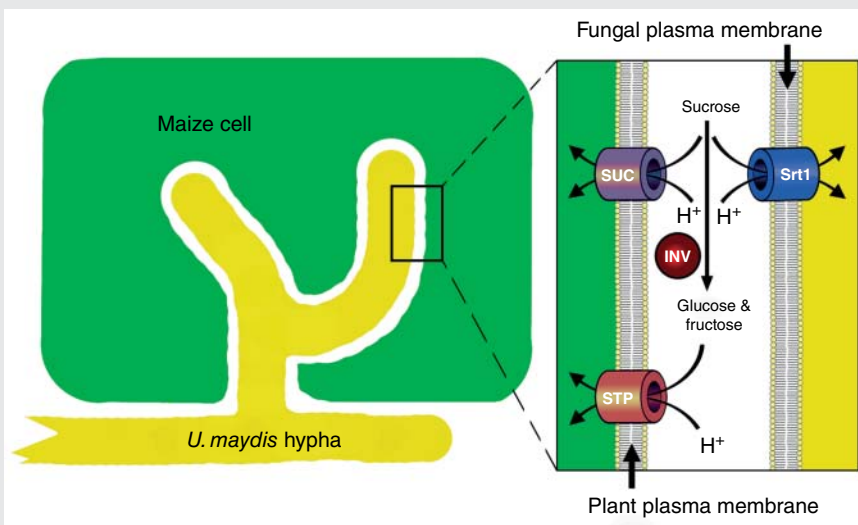


Fig. 1A Model of the bidirectional competition for extracellular sucrose at the plant/fungus interface. Plants are known to use apoplastic sucrose either via plasma membrane-localized sucrose transporters (SUC or SUT proteins) or due to the activity of extracellular invertases (INV) via membrane-localized hexose transporters (STP or MST proteins). Srt1, a high affinity sucrose H⁺-symporter, localizes to the fungal plasma membrane, and with its high substrate specificity and extremely low K_M value, it enables the fungus to efficiently use sucrose from the plant/fungus interface. Wahl *et al.* (2010). © 2010 Wahl *et al.* CC-BY-4.0.

1.2.2 Nematodes

Several hundred species of nematodes are known to feed on living plants, causing a variety of plant diseases worldwide. Plant parasitic nematodes are small: most are less than 1 mm long, although some are up to 4 mm long, with a width of 15–35 μm . They are worm-like in appearance but possess smooth, unsegmented bodies, with no appendages. In some nematode species, the female nematodes become swollen at maturity, with pear-shaped or spheroid bodies. Although most parts of the plant can be attacked by at least one species of nematode, from an economic perspective, the most important nematodes are those that feed on roots. Most plant parasitic nematodes possess a hollow stylet or spear (Fig. 1.5), although some have a solid modified spear. The stylet is used to penetrate plant cells, enabling the nematode to withdraw nutrients. Ectoparasitic nematodes, such as *Xiphenema* and *Longidorus* species, do not enter the plant root but feed by inserting the stylet into epidermal or cortical cells. In contrast, endoparasitic nematodes feed and reproduce within the plant. Sedentary endoparasites, such as root-knot and cyst nematodes, induce an amazing transformation of host cells into metabolically active transfer cells. After hatching in the soil, second-stage juveniles (J2s) move towards and penetrate plant roots. Once in the root, a root-knot nematode, such as *Meloidogyne incognita*, will move through the root intercellularly until the zone of cell division is reached.

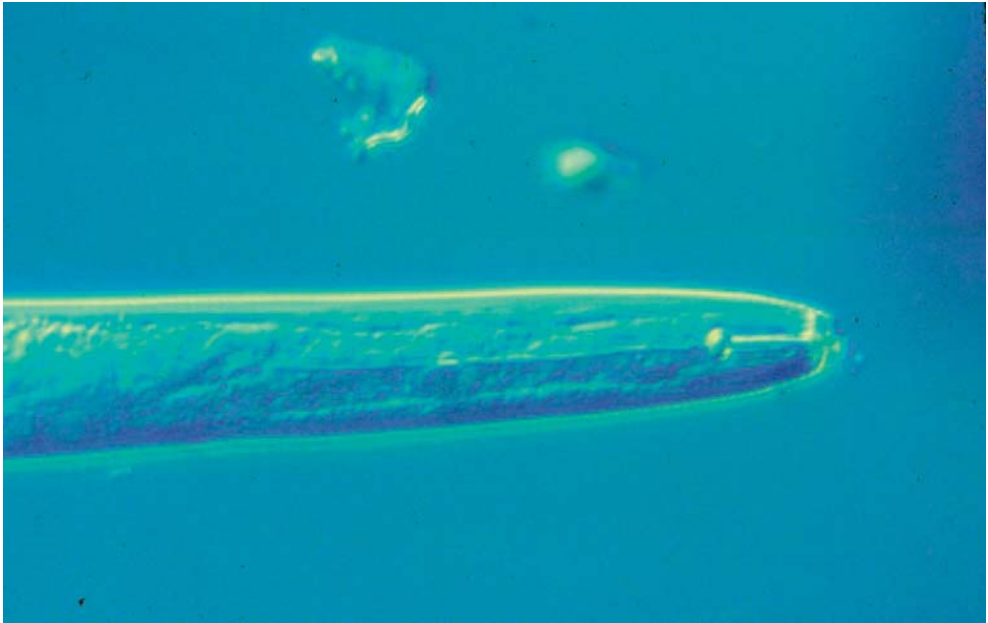


Fig. 1.5 Stylet of *Pratylenchus*, a plant-feeding lesion nematode. Soil and Water Conservation Society (SWCS) (2000). Reproduced with permission from Soil and Water Conservation Society.

In this case, the nematode injects secretions into a small number of cells, resulting in their redifferentiation into metabolically active ‘giant’ cells. Division of the surrounding cortical and pericycle cells results in localised swelling of the root and formation of the characteristic ‘root-knot’ (Fig. 1.6; Fuller *et al.*, 2008). In contrast to root-knot nematodes, cyst nematodes move through the root intracellularly, before reaching the zone of elongation, where a cell at the periphery of the vascular system is selected to become the syncytium or feeding site. In the feeding sites of both root-knot and cyst nematodes, nuclei are enlarged and endoreduplication is associated with cell enlargement (Wildermuth, 2010; also see Section 1.2.1.5). It is thought that endoreduplication is a mechanism to support the enhanced metabolic demands associated with these plant–nematode interactions. Although the feeding sites of root-knot and cyst nematodes possess different structures, both act as nutrient sinks and transfer cells, providing the nematode with the nourishment necessary for development to a mature, egg-laying female (Fuller *et al.*, 2008).

1.2.3 Insects

Amazingly, it is estimated that more than 400,000 herbivorous insect species live on some 300,000 species of vascular plant (Schoonhoven *et al.*, 2005). Among the different insect groups, herbivores are found in the Coleoptera (beetles, weevils, etc.), Lepidoptera (butterflies and moths), Hemiptera (aphids, leafhoppers, etc.), Orthoptera (grasshoppers and locusts) as well as in the Thysanoptera (thrips). There is a high degree of food specialisation among herbivorous insects, with some found on one or a few closely related plant species (monophagous), while others feed on a number of plant species (oligophagous), and yet

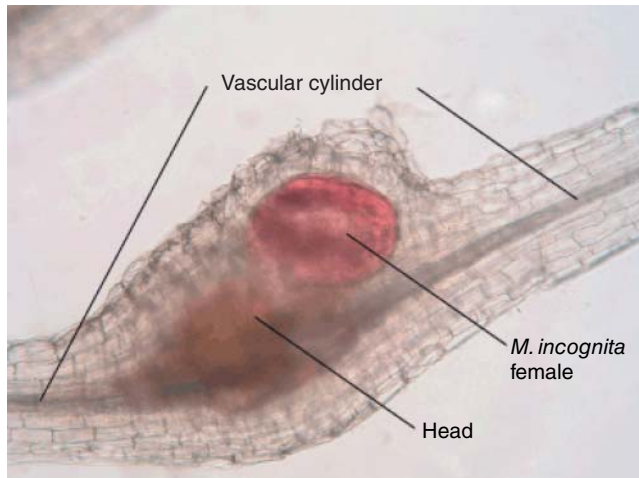


Fig. 1.6 *Arabidopsis* root being parasitized by a female *Meloidogyne incognita*, a root-knot nematode. Specialized feeding cells, termed giant cells, are induced by the nematode and are located at its head; they are connected to the vascular cylinder. Note the swelling of the root cortex around the animal and feeding cells. Fuller *et al.* (2008). Reproduced with permission of John Wiley & Sons.

others that appear to exercise little choice of plant host (polyphagous). Monophagous insects include many lepidopterous larvae, hemipterans and coleopterans, oligophagous insects include the cabbage white butterfly (*Pieris brassicae*) and the Colorado potato beetle (*Leptinotarsa decimlineata*), while the green peach aphid (*Myzus persicae*) is a good example of a polyphagous insect, feeding on members of up to 50 plant families during the summer (Schoonhoven *et al.*, 2005). However, because this classification is fairly arbitrary, it is probably more useful to distinguish between specialists (monophagous and oligophagous species) and generalists (polyphagous species).

Insects feed either by biting off and chewing plant material or by imbibing liquid from plant cells and tissues, and the two main functional groups of insect mouthparts, mandibulate and haustellate, reflect this. Mandibulate insects, which feed by biting and chewing, such as beetles and caterpillars, possess the more general type of mouthparts: (i) the labrum, a simple fused structure, often called the upper lip, and which moves longitudinally. This often contains taste sensilla, (ii) mandibles, paired structures that move at right angles to the body and which are used for biting, chewing and severing food, (iii) maxillae, paired structures that can move at right angles to the body and possess segmented palps. The maxillae help to manipulate food and guide it towards the mouth, (iv) the labium or lower lip, which is a fused structure that moves longitudinally and possesses a pair of segmented palps (Fig. 1.7). Insects that feed by imbibing liquid from the plant possess haustellate mouthparts, which can be further classified as piercing-sucking, siphoning and sponging. In piercing-sucking insects such as aphids, the mandibles and maxillae are modified to form a needle-like structure called a stylet (Fig. 1.8). This can be used to pierce the cuticle and cell wall and take up food. Some insects with haustellate mouthparts lack stylets. These insects are unable to pierce tissues and must rely on easily accessible food sources such as nectar at the base of a flower. These insects have siphoning

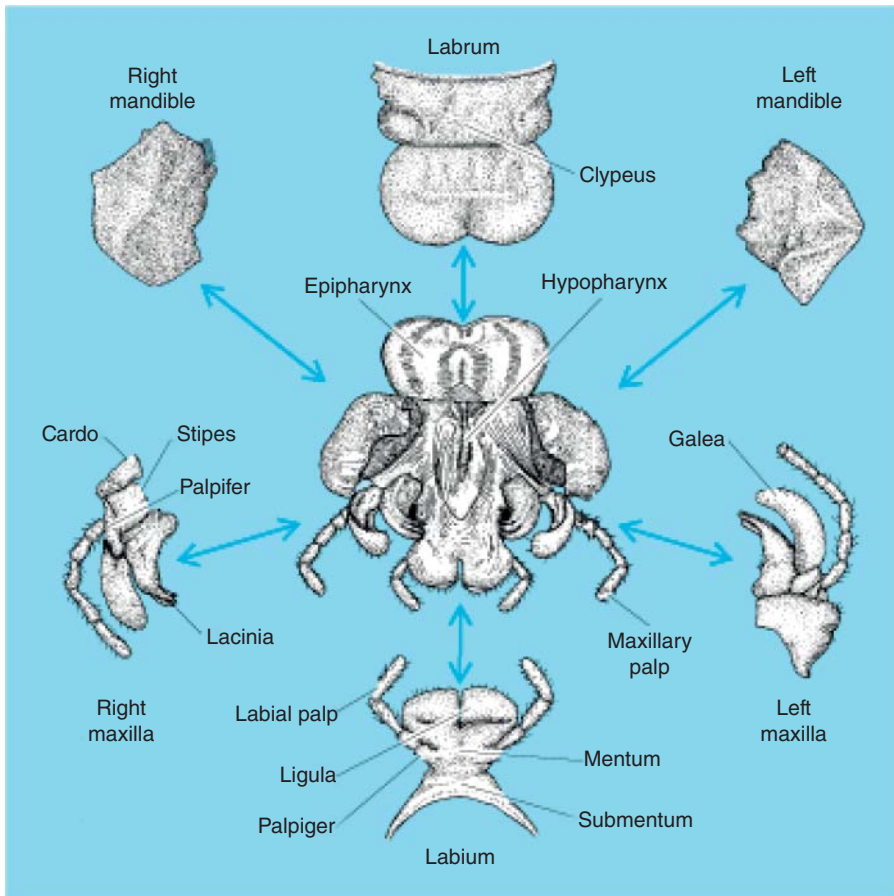


Fig. 1.7 Grasshopper mandibulate mouthparts. Metcalf *et al.* (1951). Reproduced with permission of McGraw-Hill.

mouthparts, a good example of which is the long proboscis of butterflies and moths (Fig. 1.9). Sponging mouthparts of insects such as house flies are used to sponge and suck up liquids.

Sucking insects can obtain food from several different sources in the plant. Thus, many insects belonging to the Heteroptera feed on parenchyma or xylem sap, while phloem sap is imbibed by many homopterans and psyllids. However, thrips feed on sap extracted from epidermal or parenchyma cells, using a feeding structure where several mouthparts are fused to form a mouth cone and through which the piercing organs are protruded (Schoonhoven *et al.*, 2005).

It is clear from the previous two paragraphs that there is a great deal of specialisation with regard to the feeding sites insects occupy on their hosts, with mandibulate insects such as beetles, caterpillars and grasshoppers ingesting relatively large amounts of leaf material, while insects with haustellate mouthparts imbibe liquid nourishment from the plant. However, it would be wrong to think that all mandibulate insects munch indiscriminately on leaves. Thus, leaf miners live and feed during their larval stage between the upper and lower epidermis of

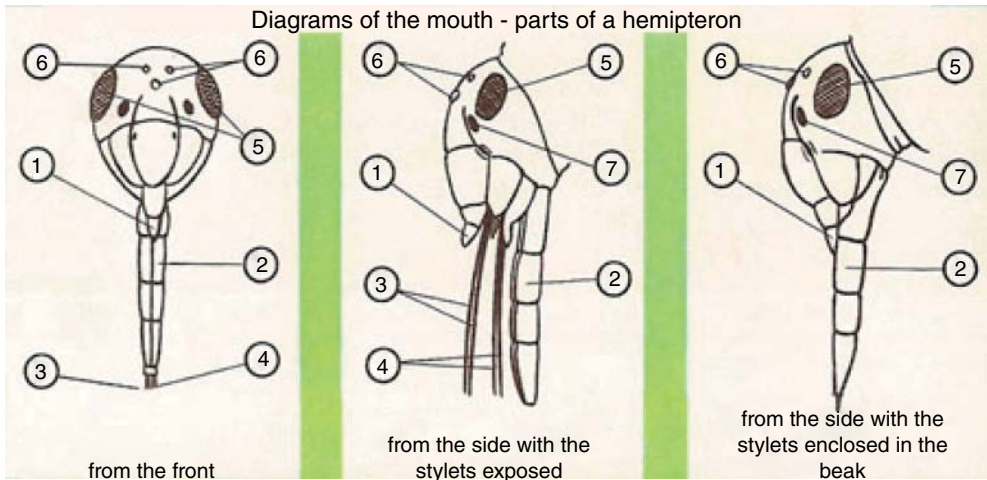


Fig. 1.8 Diagrams of the mouthparts of a Hemipteran insect. (1) upper lip or labrum (2) lower lip or labium (3) and (4) mandibles and maxillae, each having the form of bristles or stylets (5) compound eyes (6) small eyes or ocelli (7) base of the antenna. Courtesy of David Darling.

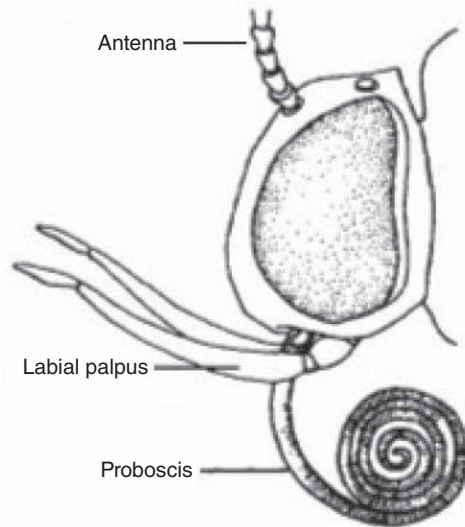


Fig. 1.9 Diagram of the siphoning mouthparts found in butterflies and some moths (Lepidoptera).

a leaf-blade, feeding on parenchymal tissues. As if this was not enough, different species of leaf miner excavate different layers of the leaf parenchyma. For example, of two hymenopterous leaf miners that attack birch leaves, *Fenusa pumila* feeds on the mesophyll, while larvae of *Messa nana* feed on palisade tissues (DeClerck & Shorthouse, 1985). In terms of root herbivory, some root-feeding insects live in the soil and eat small rootlets, others, including larvae

of cabbage root flies and carrot flies, bore directly into roots, while some aphid species pierce roots and take in liquid nourishment.

The way a plant responds to insect attack is determined, in part, by the feeding style of the attacker and by the presence of herbivore-derived elicitors in the insect's oral secretions (OS) (Rodriguez-Saona *et al.*, 2005; Felton & Tumlinson, 2008). OS from lepidopterous insects include regurgitant from the gut and saliva produced by the labial and mandibular salivary glands. These herbivore-derived elicitors, or herbivore-associated molecular patterns (HAMPs), include fatty acid conjugates such as volicitin, as well as inceptins, and can trigger biosynthesis of jasmonic acid and the release of volatile compounds (Felton & Tumlinson, 2008).

1.2.4 Parasitic plants

Parasitic plants are taxonomically and geographically diverse, comprising about 1% of the angiosperm flora (~4000 species). Interestingly, parasitic gymnosperms are considerably rarer, with only one species, *Parasitaxus usta*, identified to date (Feild & Brodribb, 2005). Broadly speaking, parasitic plants can be split into two groups, facultative parasites and obligate parasites. The former parasites possess the ability to complete their life cycle independently of the host, although their growth and reproductive potential suffer, while obligate parasitic plants cannot complete their life cycle without the host plant (Irving & Cameron, 2009). Parasitic plants can also be classified according to their site of attachment to the host plant (root or shoot) and can be defined further according to whether they contain chlorophyll. Parasitic plants containing chlorophyll are said to be hemiparasitic, while those without chlorophyll are said to be holoparasitic. Thus, *Striga hermonthica* is a root parasite that contains chlorophyll and can photosynthesise, thereby enabling it to obtain some of its resources from the host plant. Moreover, because *S. hermonthica* is dependent on the host for the period before its shoot emerges from the soil, it is an obligate hemiparasite. However, *Orobanche* species do not possess chlorophyll and derive all their resources for growth from the host plant, making them obligate holoparasites. A good example of a facultative hemiparasite is *Rhinanthus minor*, which attaches to the roots of its host and can live independently of the host plant or as a parasite (Irving & Cameron, 2009).

Parasitic plants have evolved specialist mechanisms to allow them to obtain resources from their hosts. They attach to their host using a structure known as a haustorium, which acts as a physical and physiological bridge between the parasitic plant and its host. Depending on the species of parasitic plant, contact between parasite and host can involve (i) xylem vessels of parasite and host lying adjacent to one another, (ii) direct lumenal contact between the xylem of both partners, (iii) symplastic continuity between the phloem of host and parasite or (iv) movement of either xylem or phloem solutes via specialised transfer cells into the vascular system of the parasitic plant (Fig. 1.10; Hibberd & Jeschke, 2001). In the xylem-feeding *R. minor*, the mature haustorium surrounds the host root, forming a penetration peg that forces its way through the cortex and endodermis, before being driven into the stele, gaining access to the host's vascular system (Fig. 1.11; Cameron & Seel, 2007). In the obligate parasitic plant dodder (*Cuscuta* species), the haustorium penetrates the host, producing hyphae or filaments that grow towards the host vascular system. Plasmodesmata are formed at the tip of these hyphae, creating a point of contact with the host parenchyma cells. Thereafter, parenchyma cells in the

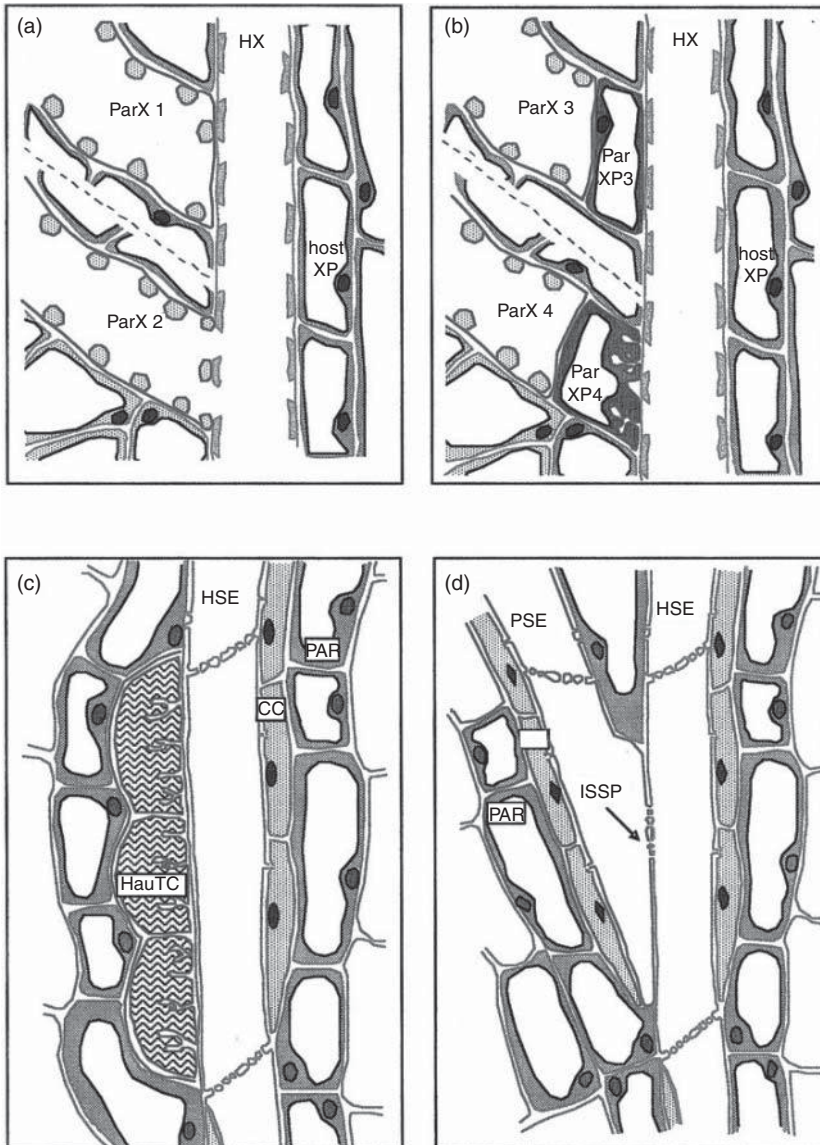


Fig. 1.10 Potential pathways via which parasitic plants could contact their hosts and access host solutes. (a) Contact between xylem of host and parasite. The xylem of parasite 1 (ParX 1) contacts the xylem of its host (HX), but there are no direct luminal connections. The xylem of the parasite 2 (ParX 2), however, forms luminal links with the host xylem. No connections are made to the host xylem parenchyma (host XP). (b) Transfer cells with fewer (ParX 3) or greater (ParX 4) degrees of cell membrane invagination of the parasite xylem parenchyma (ParXP) to facilitate solute flux, link parasite and host xylem. (c) The host sieve elements (HSE) of the phloem are lined by haustorial transfer cells (HauTC) of the parasite, which then allow unloading of host phloem solutes into the parasite haustorium. CC, companion cell; PAR, parenchyma. (d) Interspecific plasmodesmata or even interspecific sieve plates (ISSP) appear at the interface of HSE and parasite phloem sieve elements (PSE). Hibberd and Jeschke (2001). Reproduced with permission of Oxford University Press.

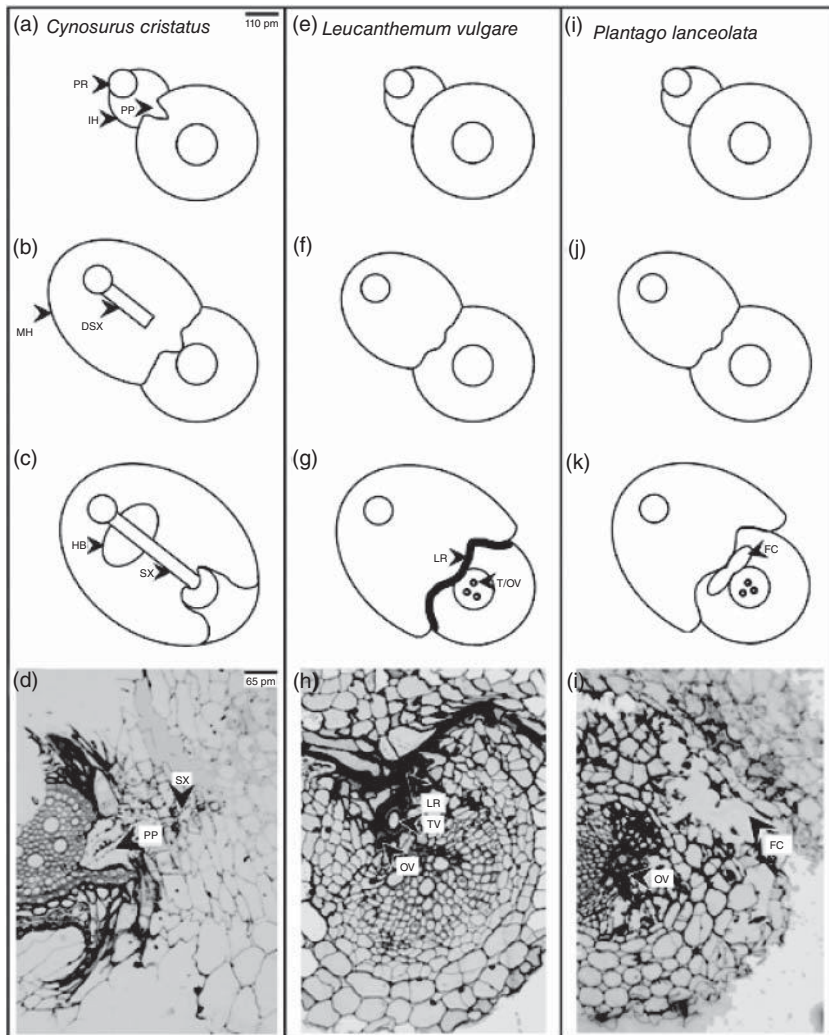


Fig. 1.11 Schematic diagram showing the ontogeny of haustoria formed by *Rhinanthus minor* on the potential hosts *Cynosurus cristatus* (a–c); *Leucanthemum vulgare* (e–g); and *Plantago lanceolata* (i–k). Transverse sections of the mature host–parasite interface with the same potential hosts are also shown (d,h,l). PR, parasite root; PP, penetration peg; IH, immature haustorium; MH, mature haustorium; DSX, developing parasite secondary xylem; HB, hyaline body; SX, fully differentiated parasite secondary xylem; LR, lignified region; FC, fragmenting host cells; T/OV, thickened/occluded host vasculature; TV, thickened host vasculature; OV, occluded host vasculature. Schematic diagrams and cross-sections of haustoria are shown on different scales; bars represent 110 µm in both cases. Cameron and Seel (2007). Reproduced with permission of John Wiley & Sons.

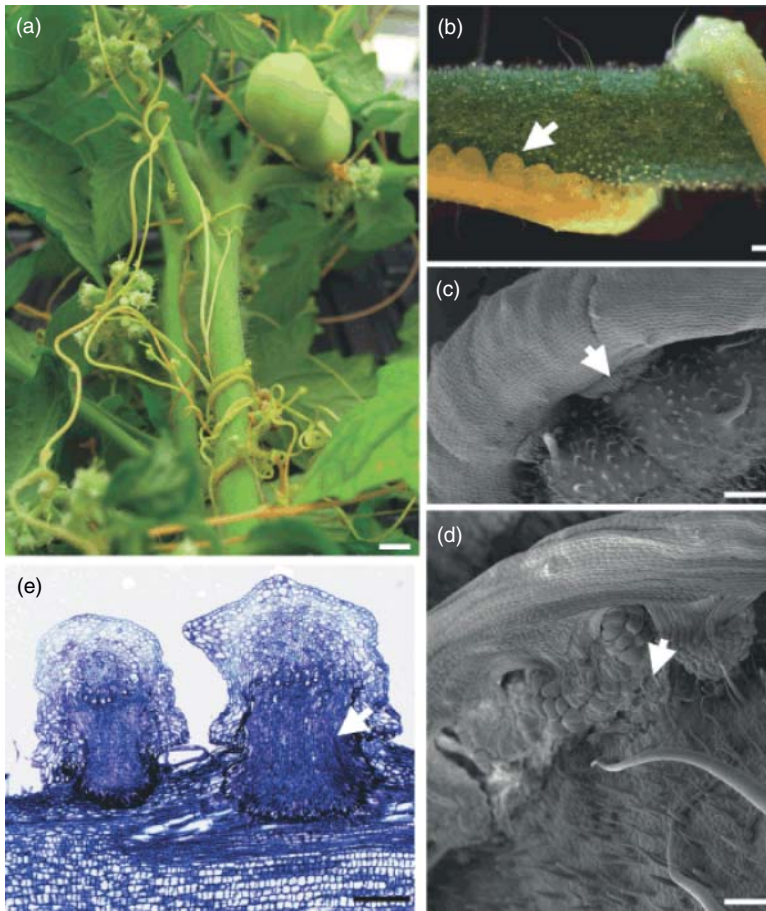


Fig. 1.12 Parasite–host interaction of tomato (*Solanum lycopersicum*) and dodder (*Cuscuta pentagona*). (a) Dodder parasitizing a 7-week-old tomato plant, 4 week after attachment. Bar, 5 mm. (b) Haustorium formation on tomato petiole (arrow). Bar, 500 μ m. (c) Scanning electron microscope (SEM) image of young haustoria (arrow) in dodder–tomato interaction. (d) SEM image of mature haustoria (arrow) detaching from tomato leaf demonstrating the interactions between the two organisms. (e) A cross-section of two adjacent haustoria establishing an internal connection (arrow) with the tomato host leaving a penetration fissure behind. Bar, 500 μ m. David-Schwartz *et al.* (2008). Reproduced with permission of John Wiley & Sons.

parasite haustorium differentiate into xylem and phloem elements, which then associate with the host vascular system. This results in the formation of phloem–phloem and xylem–xylem connections between the parasite and its host (Fig. 1.12; David-Schwartz *et al.*, 2008). Such vascular connections were shown to be continuous and functional by following the transfer of various molecules from the host to the parasitic plant. For example, labelled amino acids and sugars were found to move from the host into *Cuscuta*, while green fluorescent protein was demonstrated to cross the host–dodder vascular junction (Tsivion, 1978; Haupt *et al.*, 2001; Birschwilks *et al.*, 2006).

In *Cuscuta reflexa* and *Orobancha crenata*, both of which lack roots, all minerals must come from the host, and in both cases, most are derived via the phloem rather than the xylem. Because *Orobancha* lacks chlorophyll and therefore cannot photosynthesise, all of its carbon must also come from the host plant. Interestingly, although *Cuscuta* retains functional photosynthetic apparatus in a ring of cells around the stele, nearly all of its carbon also comes from the host (Jeschke *et al.*, 1994; Hibberd & Jeschke, 2001). Xylem feeders tend to be hemiparasites, using the xylem of the host plant to bolster their own resources. However, although they were thought to be largely self-sufficient for carbon, it is clear that hemiparasites such as *R. minor*, which can photosynthesise, also obtain carbon from their hosts. Indeed, the Australian hemiparasite *Olex phyllanthi* was found to abstract roughly 27% of recent photosynthate from its host (Tennakoon *et al.*, 1997). Facultative hemiparasites also obtain substantial quantities of nitrogen from their hosts, with *O. phyllanthi* taking 56% of newly fixed nitrogen from its leguminous host, *Acacia littorea* (Tennakoon *et al.*, 1997). As mentioned previously, the obligate hemiparasite *S. hermonthica* is entirely reliant on its host for the 4–6-week period when the young plant remains underground. Once *S. hermonthica* emerges from the soil and can photosynthesise, it becomes less reliant on the host for carbon. However, since its photosynthetic rates are very low, it still obtains up to 33% of its carbon from the host (Press *et al.*, 1987).

1.3 SYMPTOMS EXHIBITED BY PLANTS FOLLOWING ATTACK

As we have seen in the previous sections, different organisms have different approaches to attacking or entering a plant and obtaining the nourishment necessary for continued growth and development. The method of interaction with the plant is likely to affect its functioning, resulting in the appearance of symptoms. A symptom is a visible or measurable sign that the plant is not functioning normally. Sometimes, a symptom can be diagnostic for a specific disease, for example, but more often, a given symptom on a host might be due to one or more of a variety of causes.

The major symptoms exhibited by plants attacked by pathogens, pests, nematodes and parasitic plants are listed in Table 1.3. Associated with these symptoms are the functions likely to be affected, although, as pointed out by Lucas (1998), this means of classification is arbitrary and non-specific. He uses the example of permanent wilting, which could be the result of a blockage in the host xylem, destruction of root tissues or increased transpiration. Just how important a particular symptom is depends on a number of factors, including the stage of plant growth or development and the duration and severity of the symptom. This can be illustrated by two examples, chlorosis and necrosis. Chlorosis, or yellowing of leaves, is associated with impairment of photosynthesis (see Chapter 3). Although chlorosis in young cereal plants will reduce rates of photosynthesis, this is unlikely to exert much effect on grain yield, as most assimilates required for grain filling come from the flag leaf and ear tissues. Necrosis, or cell and tissue death, in the stem of a seedling, could completely disrupt transport of assimilates from leaves to roots and water and nutrients from roots to shoot, resulting in plant death. However, necrosis in the stem of a mature, woody perennial might result in the loss of a branch or twig, rather than the whole plant.

Table 1.3 Symptoms caused by pathogens, herbivores and parasitic plants in relation to function in higher plants.

	Vegetative organs			Reproductive organs	
	Roots	Stems	Leaves	Flowers, fruit	Seeds, seedlings
Functions	Uptake Transport Anchorage	Support Transport	Photosynthesis Gas exchange Transpiration	Fertilization Development	Survival Germination
Symptoms	Necrosis Hypertrophy Hyperplasia Excessive branching	Necrosis Etiolation Gall formation Excessive branching Lodging	Chlorosis Pigment changes Necrosis Wilting Epinasty Hypertrophy Abscission Gall formation	Inhibition Substitution Necrosis	Necrosis
Pathogen/ Pest/parasitic plant examples	Root rots Club root Rhizomania Root knot nematodes	Heart rots Foot rots Cankers Crown gall Witch's broom Bakanae disease Cereal eyespot	Mosaic Leaf spots Blight Leaf roll/curl Vascular wilts Leaf cast Coffee rust Cynipid wasp larvae <i>Striga</i> infection	Choke Ergot Anther smut Storage rots	Seed decay Damping-off

Source: Adapted from Lucas (1998). Reproduced with permission of John Wiley & Sons.

1.4 CONCLUSIONS

As we have seen in this chapter, plants are attacked by a great many organisms, which use a variety of approaches to obtain the nourishment locked away within their tissues. The physical damage caused can be minor or can be quite considerable. In addition, even if little physical damage is caused, physiological function can be impaired. The combined effects of physical damage and disruption of plant function can be serious, reducing plant growth and reproduction and, in some cases, leading to death of the whole plant. This can have far-reaching consequences for plants in both natural and managed systems, resulting in changes in plant populations and loss of crop yield and quality. These aspects are covered in the next chapter.

RECOMMENDED READING

- Agrios GN, 2005. *Plant pathology*, third edition. London: Elsevier Academic Press.
- Felton GW, Tumlinson JH, 2008. Plant-insect dialogues: complex interactions at the plant-insect interface. *Current Opinion in Plant Biology* **11**, 457–463.
- Fuller VL, Lilley CJ, Urwin PE, 2008. Nematode resistance. *New Phytologist* **180**, 27–44.
- Irving LJ, Cameron DD, 2009. You are what you eat: interactions between root parasitic plants and their hosts. *Advances in Botanical Research* **50**, 87–138.

Schoonhoven LM, van Loon JJA, Dicke M, 2005. *Insect-plant biology*. Oxford: Oxford University Press.
 Walters DR, 2011. *Plant defense: warding off attack by pathogens, herbivores, and parasitic plants*. Oxford: Wiley-Blackwell.

REFERENCES

- Agrios GN, 2005. *Plant pathology*, third edition. London: Elsevier Academic Press.
- Birschwilks M, Haupt S, Hofius D, Neumann S, 2006. Transfer of phloem-mobile substances from the host plants to the holoparasite *Cuscuta* sp. *Journal of Experimental Botany* **57**, 911–921.
- Cameron DD, Seel WE, 2007. Functional anatomy of haustoria formed by *Rhinanthus minor*: linking evidence from histology and isotope tracing. *New Phytologist* **174**, 412–419.
- Chandran D, Inada N, Hather G, Kleindt CK, Wildermuth MC, 2010. Laser microdissection of *Arabidopsis* cells at the powdery mildew infection site reveals site-specific processes and regulators. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 460–465.
- David-Schwartz R, Runo S, Townsley B, Machuka J, Sinha N, 2008. Long-distance transport of mRNA via parenchyma cells and phloem across the host-parasite junction in *Cuscuta*. *New Phytologist* **179**, 1133–1141.
- DeClerck RA, Shorthouse JD, 1985. Tissue preference and damage by *Fenusa pusilla* and *Mesa nana* (Hymenoptera: Tenthredinidae), leaf mining sawflies on white birch (*Betula papyrifera*). *Canadian Entomologist* **117**, 351–362.
- Dulermo T, Rasle C, Chinnici G, Gout E, Bligny R, Cotton P, 2009. Dynamic carbon transfer during pathogenesis of sunflower by the necrotrophic fungus *Botrytis cinerea*: from plant hexoses to mannitol. *New Phytologist* **183**, 1149–1162.
- Ehness R, Ecker M, Godt DE, Roitsch T, 1997. Glucose and stress independently regulate source and sink metabolism and defense mechanisms via signal transduction pathways involving protein phosphorylation. *Plant Cell* **9**, 1825–1841.
- Feild TS, Brodribb TJ, 2005. A unique mode of parasitism in the conifer coral tree *Parasitaxus ustus* (Podocarpaceae). *Plant, Cell and Environment* **53**, 39–43.
- Felton GW, Tumlinson JH, 2008. Plant-insect dialogues: complex interactions at the plant-insect interface. *Current Opinion in Plant Biology* **11**, 457–463.
- Firrao G, Gibb K, Streten C, 2005. Short taxonomic guide to the genus ‘*Candidatus Phytoplasma*’. *Journal of Plant Pathology* **87**, 249–263.
- Fuller VL, Lilley CJ, Urwin PE, 2008. Nematode resistance. *New Phytologist* **180**, 27–44.
- Haupt S, Oparka KJ, Sauer N, Neumann S, 2001. Macromolecular trafficking between *Nicotiana tabacum* and the holoparasite *Cuscuta reflexa*. *Journal of Experimental Botany* **52**, 556–562.
- Hibberd JM, Jeschke WD, 2001. Solute flux into parasitic plants. *Journal of Experimental Botany* **52**, 2043–2049.
- Irving LJ, Cameron DD, 2009. You are what you eat: interactions between root parasitic plants and their hosts. *Advances in Botanical Research* **50**, 87–138.
- Jeschke WD, R  th N, B  umel P, Czygan F-C, Proksch P, 1994. Modelling the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L.: I. Methods for estimating net flows. *Journal of Experimental Botany* **45**, 791–800.
- Kneale J, Farrar JF, 1985. The localisation and frequency of haustoria in colonies of brown rust on barley leaves. *New Phytologist* **101**, 495–505.
- Kocal N, Sonnewald U, Sonnewald S, 2008. Cell wall bound invertase limits sucrose export and is involved in symptom development and inhibition of photosynthesis during compatible interaction between tomato and *Xanthomonas campestris* pv *vesicatoria*. *Plant Physiology* **148**, 1523–1536.
- Lewis DH, Smith DC, 1967. Sugar alcohols (polyols) in fungi and green plants. I. Distribution, physiology and metabolism. *New Phytologist* **66**, 143–184.
- Lucas JA, 1998. *Plant pathology and plant pathogens*, third edition. Oxford: Blackwell Publishing Ltd.
- Maniloff J, 2002. Phylogeny and evolution. In: Razin S, Herrmann R, eds. *Molecular biology and pathogenicity of mycoplasmas*. New York: Kluwer Academic/Plenum Publishing, pp. 31–43.
- Nadwodnik J, Lohaus G, 2008. Subcellular concentrations of sugar alcohols and sugars in relation to phloem translocation in *Plantago major*, *Plantago maritime*, *Prunus persica*, and *Apium graveolens*. *Planta* **227**, 1079–1089.

- Newton AC, Fitt BDL, Atkins SD, Walters DR, Daniell TJ, 2010. Pathogenesis, parasitism and mutualism in the trophic space of plant-microbe interactions. *Trends in Microbiology* **18**, 365–373.
- O'Connell RJ, Panstruga R, 2006. Tête à tête inside a plant cell: establishing compatibility between plants and biotrophic fungi and oomycetes. *New Phytologist* **171**, 699–718.
- Parker D, Beckmann M, Zubair H, Enot DP, Caracul-Rios Z, *et al.*, 2009. Metabolomic analysis reveals a common pattern of metabolic re-programming during invasion of three host plant species by *Magnaporthe grisea*. *The Plant Journal* **59**, 723–737.
- Polidori F, Ceccaroli P, Saltarelli R, Guescini M, Menotta M, *et al.*, 2007. Hexose uptake in the plant symbiotic ascomycete *Tuber borchii* Vittadini: biochemical features and expression pattern of the transporter TBHXT1. *Fungal Genetics and Biology* **44**, 187–198.
- Press MC, Shah N, Tuohy JM, Stewart GR, 1987. Carbon isotope ratios demonstrate carbon flux from C4 host to C3 parasite. *Plant Physiology* **85**, 1143–1145.
- Rodriguez-Saona C, Chalmers J, Raj S, Thaler J, 2005. Induced plant responses to multiple damagers: differential effects on an herbivore and its parasitoid. *Oecologia* **143**, 566–577.
- Roitsch T, Balibrea ME, Hoffmann M, Proels R, Sinha AK, 2003. Extracellular invertase: key metabolic enzyme and PR protein. *Journal of Experimental Botany* **54**, 513–524.
- Rolland F, Bacna-Gonzalez E, Sheen J, 2006. Sugar sensing and signalling in plants: conserved and novel mechanisms. *Annual Review of Plant Biology* **57**, 675–709.
- Scholes JD, Lee PJ, Horton P, Lewis DH, 1994. Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. *New Phytologist* **126**, 213–222.
- Schoonhoven LM, van Loon JJA, Dicke M, 2005. *Insect-plant biology*. Oxford: Oxford University Press.
- Struck C, Ernst M, Hahn M, 2002. Characterization of a developmentally regulated amino acid transporter (AAT1p) of the rust fungus *Uromyces fabae*. *Molecular Plant Pathology* **3**, 23–30.
- Tabler M, Tsagris M, 2004. Viroids: petite RNA pathogens with distinguished talents. *Trends in Plant Science* **9**, 339–348.
- Tang X, Rolfe SA, Scholes JD, 1996. The effect of *Albugo candida* (white blister rust) on the photosynthetic and carbohydrate metabolism of leaves of *Arabidopsis*. *Plant, Cell and Environment* **19**, 967–975.
- Tennakoon K, Pate JS, Fineran A, 1997. Growth and partitioning of C and fixed N in the shrub legume *Acacia littorea* in the presence or absence of the root hemiparasite *Oxalophyllanthii*. *Journal of Experimental Botany* **48**, 1047–1060.
- Tsivion Y, 1978. Loading of assimilates and some sugars into the translocation system of *Cuscuta*. *Australian Journal of Plant Physiology* **5**, 851–857.
- Voegelé RT, Mendgen K, 2003. Rust haustoria: nutrient uptake and beyond. *New Phytologist* **159**, 93–100.
- Voegelé RT, Struck C, Hahn M, Mendgen K, 2001. The role of haustoria in sugar supply during infection of broad bean by the rust fungus *Uromyces fabae*. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 8133–8138.
- Voegelé RT, Hahn M, Lohaus G, Link T, Heiser I, Mendgen K, 2005. Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiology* **137**, 190–198.
- Voegelé RT, Wirsal S, Möll U, Lechner M, Mendgen K, 2006. Cloning and characterisation of a novel invertase from the obligate biotroph *Uromyces fabae* and analysis of expression patterns of host and pathogen invertases in the course of infection. *Molecular Plant-Microbe Interactions* **19**, 625–634.
- Wahl R, Wippel K, Goos S, Kämper J, Sauer N, 2010. A novel high-affinity sucrose transporter is required for virulence of the plant pathogen *Ustilago maydis*. *Plos Biology* **8** (2), 1–11.
- Walters DR, 2011. *Plant defense: warding off attack by pathogens, herbivores, and parasitic plants*. Oxford: Wiley-Blackwell.
- Walters DR, McRoberts N, Fitt BDL, 2008. Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biological Reviews* **83**, 79–102.
- Wharton PS, Julian AM, O'Connell RJ, 2001. Ultrastructure of the infection of *Sorghum bicolor* by *Colletotrichum sublineolum*. *Phytopathology* **91**, 149–158.
- Wildermuth MC, 2010. Modulation of host nuclear ploidy: a common plant biotroph mechanism. *Current Opinion in Plant Biology* **13**, 449–458.

2 Growth, Development and Yield of Infected and Infested Plants and Crops

2.1 INTRODUCTION

The *raison d'être* of parasitism and herbivory is to obtain nourishment, thereby allowing the attacking organism to grow, develop and reproduce. It stands to reason therefore that the loss of plant resources to the attacker will have an effect on the ability of the plant to service its own growth. Moreover, as we have seen in Chapter 1, the manner in which the attacker obtains food from the host plant, and the resulting symptoms of the attack, will also have an effect on the functioning of the plant, which in turn, will affect plant growth and development. Although viruses do not obtain nourishment from the plant, host resources and cellular machinery are used in the synthesis of new virus particles, disrupting host cell functioning in the process.

In this chapter, we examine the effects of pathogens, pests and parasitic plants on the growth, development and reproduction of plants. Such effects can have serious consequences agriculturally, ecologically and socially.

2.2 EFFECTS OF PATHOGENS ON GROWTH, DEVELOPMENT AND YIELD

The magnitude and severity of the effects of microbial pathogens on their hosts are out of all proportion to their size. It is staggering to think that microscopic organisms can destroy crops and cause great human suffering, but as we shall see later in this chapter, microbial pathogens of plants have exerted profound effects on the course of human history.

Pathogens might reduce plant growth, and ultimately yield, by destruction of leaf or root tissue or by causing leaves to become chlorotic. Plant reproduction and yield might also be affected by direct effects on flowers, for example. Such effects on plant growth are relatively easy to understand because of the underlying effects on host physiology, such as reduced photosynthesis, impaired uptake and transport of water and minerals or perturbation of normal reproductive development. However, pathogen infection can also lead to abnormal growth of plant tissues and organs. Good examples include clubroot of Brassicas, caused by the

plasmodiophoromycete pathogen *Plasmodiophora brassicae* and crown gall of many hosts, caused by the bacterium *Agrobacterium tumefaciens*. Reductions in plant growth and yield, and abnormal effects on plant growth and development, are dealt with in the following sections. The mechanisms underlying these effects are dealt with in later chapters.

Infection by plant pathogens commonly results in reduced vegetative growth both in wild species and in crop plants, although the mechanisms responsible for growth reductions are likely to differ depending on the mode of nutrition and growth habit of the pathogen. For example, although growth reductions might be traced back to reduced photosynthetic rates, the underlying mechanisms are likely to depend on the particular plant–pathogen interaction. Thus, reductions in photosynthesis resulting from infection by biotrophic fungal pathogens such as rusts and powdery mildews are likely to be related to subtle reprogramming of host metabolism, whereas photosynthetic reductions resulting from infection by necrotrophic foliar pathogens are likely to be due, at least in part, to the loss of leaf area. In contrast, pathogens that destroy root tissue, such as *Pythium* spp., or those that live in association with the host vascular system, such as *Verticillium* and *Fusarium*, will disrupt water uptake and transport by the host, with consequences for photosynthesis.

2.2.1 Biotrophic pathogens

Infection by biotrophic pathogens such as rusts and powdery mildews commonly results in reduced vegetative growth, both in crop plants and in wild species. Infection of crop plants by such pathogens can modify dry weight distribution, leading to greater reductions in root growth than shoot growth (Last, 1962; Doodson *et al.*, 1964; Walters & Ayres, 1981). Ultimately, infection can also lead to reductions in yield, with powdery mildew on barley and yellow rust on wheat, both reducing the number of grains per ear and the size of individual grains (Doodson *et al.*, 1964; Carver & Griffiths, 1981). However, the timing and severity of infection can influence which components of yield are most affected. Thus, in barley, early attack by powdery mildew is most damaging to plants, mainly affecting the number of fertile tillers (Scott & Griffiths, 1980), although the number of grains per year and grain size can also be reduced. In contrast, if powdery mildew infection occurs late in the season, yield reductions are usually attributed to reductions in grain size.

As indicated previously, infection can also reduce the growth of wild plants. For example, rust infection of groundsel (*Senecio vulgaris*) reduced growth of all plant organs, but unlike crop plants, growth of the individual plant parts was reduced to a similar extent, with little change in the partitioning of dry weight in the plant (Fig. 2.1; Paul & Ayres, 1987). If reduced root growth limits the performance of infected plants, it is possible that the stability of partitioning to the roots might be important in moderating the impact of infection on plants such as groundsel under field conditions (Paul & Ayres, 1987). Rust infection also reduced the reproductive capacity in groundsel, with infected plants producing fewer flowers and, as a result, fewer seed. Moreover, the longevity of plants was also affected by rust infection, with infected plants dying earlier than their uninfected counterparts (Fig. 2.2; Paul & Ayres, 1986a,b). These effects of rust infection, if repeated over several seasons, would have a significant effect on the population size of groundsel.

Virus infections can also lead to considerable yield losses under favourable conditions. Barley Yellow Dwarf (BYD) is the most common and serious disease of cereal crops worldwide, causing 1–3% yield losses annually in the United States (Burnett & Mezzalama, 1990). However, losses under favourable conditions can be considerably higher. In a study of the

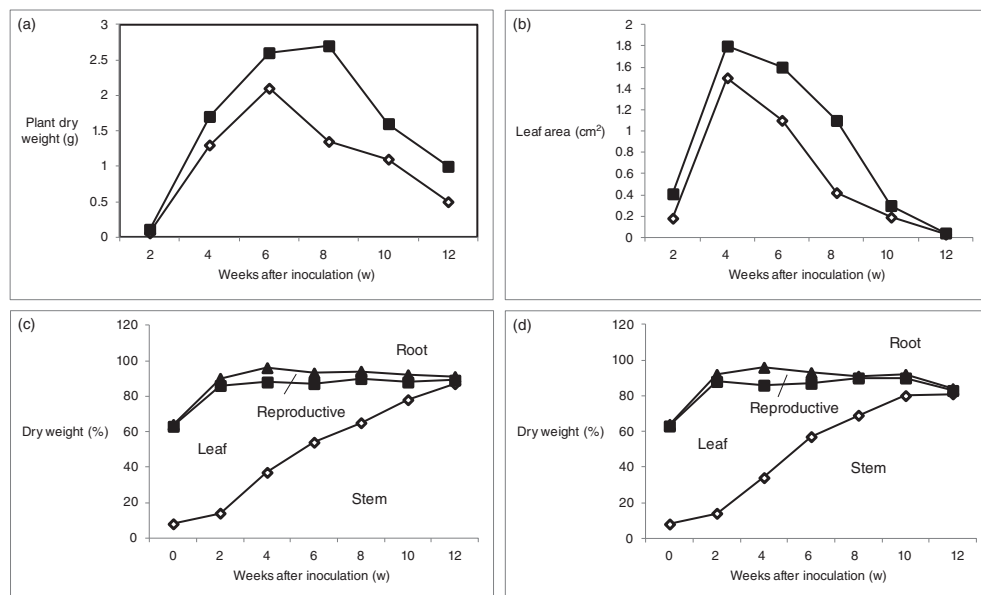


Fig. 2.1 Growth and partitioning of dry weight of groundsel (*Senecio vulgaris*) infected by rust (*Puccinia lagenophorae*). (a) Total plant dry weight and (b) leaf area of surviving uninoculated (closed symbols) and inoculated plants (open symbols). Patterns of dry weight partitioning in uninoculated (c) and inoculated plants (d). Values in (c) and (d) are percentage dry weight in leaf, stem, root and reproductive organs. Paul and Ayres (1987). Reproduced with permission of John Wiley & Sons.

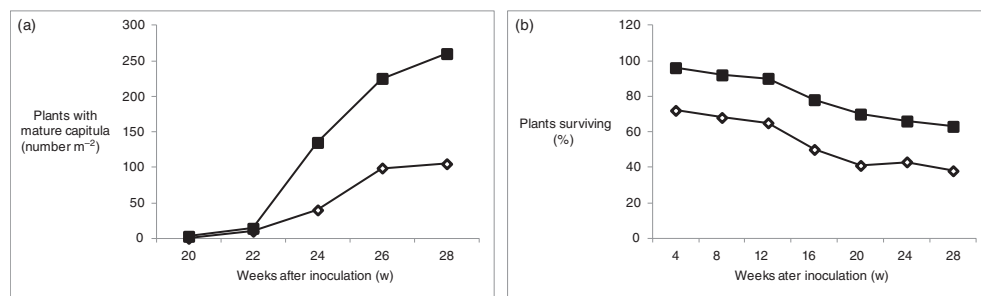


Fig. 2.2 Reproduction and survival of groundsel (*Senecio vulgaris*) infected by rust (*Puccinia lagenophorae*). (a) Changes in the number of plants with mature capitula in control populations (closed symbols) and populations inoculated with rust (open symbols). Paul and Ayres (1986a). Reproduced with permission of John Wiley & Sons. (b) Changes in the percentage of the original groundsel population surviving with time in controls (closed symbols) and following inoculation with rust (open symbols). Paul and Ayres (1986b). Reproduced with permission of John Wiley & Sons.

effects of *barley yellow dwarf virus* (BYDV) on three cultivars of malting barley, Edwards *et al.* (2001) found that yields were reduced between 8.5% and 38% over 2 years, and grain quality was also negatively affected. Another virus infecting cereals is *rice black-streaked dwarf virus* (RBSDV). It was first reported in Japan in 1952 (Kuribayashi & Shinkai, 1952) and in China in 1963 (Ruan *et al.*, 1984) but declined in importance until the mid-1990s. However, since 1996,

the disease has re-emerged and in the Zhejiang province has been estimated to cause losses of circa 120,000 tonnes of rice grain each year (Wang *et al.*, 2009). An intensive survey and monitoring of the emergence of the disease were carried out in this province during the period 1995–2007. Analysis of the data revealed a highly significant relationship between disease incidence and yield loss, with every 1% increase in disease incidence leading to yield losses of 0.80% and 0.92% for early *indica* and late *japonica* rice, respectively (Wang *et al.*, 2009).

2.2.2 Necrotrophic pathogens

Plant growth and yield can also be reduced after infection by necrotrophic pathogens. For example, in barley infected with *Pyrenophora teres*, dry weights of root and shoot, as well as leaf area, were reduced, although root growth was affected to a greater extent than shoot growth (Fig. 2.3; Jordan *et al.*, 1985). Infection also reduced grain yield. In this case, the effect was dependent on the stage of plant growth when it was inoculated with the pathogen. Grain yield was reduced substantially after inoculation with *P. teres* at growth stage 39 (flag leaf blade visible), which resulted in a rapid loss of green leaf tissue. This should not be surprising,

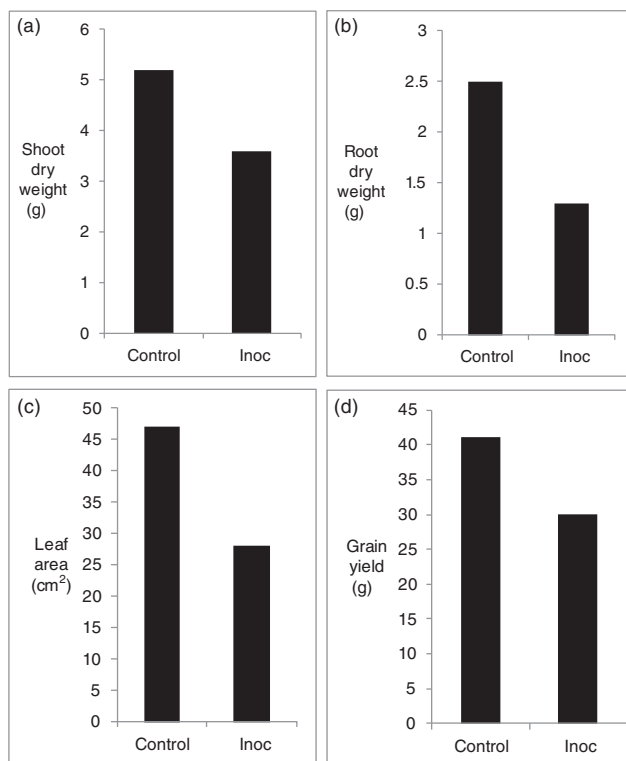


Fig. 2.3 Effect of *Pyrenophora teres* on growth and yield of winter barley in a glasshouse experiment. (a) shoot dry weight (b) root dry weight (c) total leaf area (d) grain yield. Plants were inoculated with the pathogen at three growth stages, GS11 + GS13 + GS30. Control plants were not inoculated. For shoot and root dry weights and total leaf area, measurements were made at GS31. Jordan *et al.* (1985). Reproduced with permission of John Wiley & Sons.

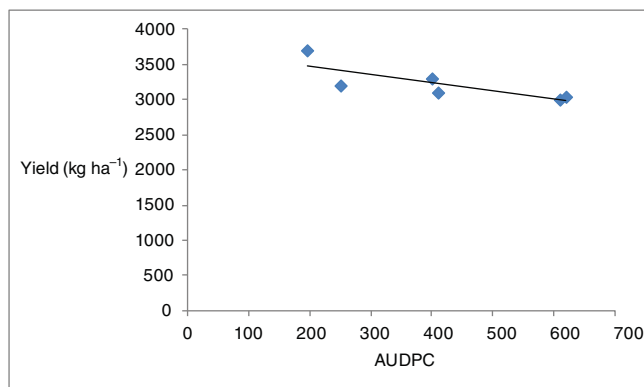


Fig. 2.4 Effect of *Mycosphaerella* blight on yield of pea: relationship between AUDPC of *Mycosphaerella* blight and yield in a pea crop grown in Edmonton, Alberta, Canada, in 2003. Adapted from Su *et al.* (2006) with permission of Verlag Eugen Ulmer and S-F Hwang.

because in barley, the flag leaf and the leaf below it are major contributors of assimilate for grain yield. Indeed, substantial yield losses in barley have also been associated with infection of these two leaves with powdery mildew and the leaf scald pathogen, *Rhynchosporium secalis* (now *R. commune*) (Large & Doling, 1962; James, 1967).

Mycosphaerella blight is a serious disease of field peas. Caused by *Mycosphaerella pinodes*, it reduces both the number of seeds and seed weight per plant and can lead to crop losses of up to 50% (Garry *et al.*, 1998; Xue & Warkentin, 2001). Work on *Mycosphaerella* blight on field peas in Western Canada in 2002 and 2003 demonstrated a linear relationship between yield loss and final disease severity or area under the disease progress curve (AUDPC) (Fig. 2.4; Su *et al.*, 2006). However, such relationships between yield loss and disease severity do not always exist. For example, *Phaeoisariopsis griseola* causes angular leaf spot on French bean, *Phaseolus vulgaris*. Together with rust caused by *Uromyces appendiculatus*, they represent important diseases of the crop in various parts of the world. Angular leaf spot can lead to partial defoliation of plants, while rust does not cause defoliation but can reduce rates of photosynthesis. In a comparative study, surprisingly, yield was not related to AUDPC for either disease (de Jesus Junior *et al.*, 2001). Apparently, although both diseases reduced yield, there were significant differences in yield reductions between different years.

2.2.3 Vascular wilt pathogens

Vascular wilt pathogens include the fungi *Verticillium* and *Fusarium*, and bacteria such as *Ralstonia solanacearum*. They colonise the xylem vessels of their hosts, which can become blocked by fungal mycelia or bacterial cells, as well as by the polysaccharides and pectolytic enzymes secreted by these pathogens. Vessels can also become blocked by the gums, mucilages and tyloses produced by the host in response to pathogen invasion. Blockage of xylem vessels can reduce water flow considerably, resulting in water stress. Pathogen-induced water stress has been implicated in the growth reductions observed in plants infected with vascular wilt pathogens. For example, in sunflower infected with *V. dahliae*, reduced shoot biomass and leaf area was considered to be the result of pathogen-induced water stress

Table 2.1 The relative effects of three strains of *Verticillium albo-atrum* on vascular colonisation, tylose production and leaf area of four tomato cultivars.

Host/pathogen strain	Vascular colonisation	Tylosis	Reduction in leaf area (%)
Bonny best			
T	++	++	93.2
HP	++	+	68.2
HF	++	+	62.1
Potentate			
T	++++	—	91.4
HP	++	+++	49.2
HF	++	+++	48.4
Loran blood			
T	+	+	4.4
HP	+	+++	66.8
HF	+	+++	53.7
Moscow			
T	++++	++	nil
HP	+	+++++	58.0
HF	+	+++	29.8

T, tomato strain; HP, hop progressive strain; HF, hop fluctuating strain.

+++++, very heavy; +++++, heavy; +++, medium; ++, light; +, very light; —, absent.

Source: Adapted from Pegg and Dixon (1969). Reproduced with permission of John Wiley and Sons.

(Sadras *et al.*, 2000). However, the situation can be less clear cut in other host–pathogen interactions. Thus, in tomato inoculated with *V. albo-atrum*, reductions in leaf area were not always associated with the production of tyloses, and observed effects were dependent on the interaction of particular host cultivars and pathogen strains (Table 2.1; Pegg & Dixon, 1969). Assessing the effects of vascular blockage is further complicated because these fungi also produce toxins, which can exert physiological effects in the host plant.

Chickpea is the world's third most important pulse crop after beans and peas. Production can be seriously affected by Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *ciceris*, in most chickpea growing regions, with annual losses of up to 15%, although complete crop losses have been reported (Trapero-Casas & Jiménez-Díaz, 1985; Halila & Strange, 1996). In a study of 108 epidemics of Fusarium wilt on chickpea during the period 1986–1989, the overall yield loss was attributed mainly to a reduction in the number of seeds per plant and, to a lesser extent, to reduced mean seed weights (Navas-Cortéz *et al.*, 2000). This study revealed a significant relationship between chickpea seed yield and severity of Fusarium wilt.

2.3 EFFECTS OF NEMATODES ON GROWTH, DEVELOPMENT AND YIELD

Nematodes can cause substantial reductions in plant growth. Although the direct effect of most nematodes is on the roots, because they can destroy root tissue, root function can be compromised and growth of the whole plant can be affected. For example, in banana, infestation with a mixture of migratory endoparasitic nematodes reduced root and shoot growth. Interestingly,

resistant varieties partitioned more biomass to roots than shoots, thereby contributing to greater root mass and a greater number of primary roots than less resistant varieties (Kalorizou *et al.*, 2007a,b). Effects of nematodes on root growth can be rapid. Potato root growth was reduced within 24 hours following inoculation of juvenile nematodes (*Globodera pallida*) directly onto root tips, although the magnitude of the growth reductions was dependent on host genotype (Arnitzen *et al.*, 1994).

Nematode infection can lead not only to reductions in growth, but also in yield. In spring wheat in the north western United States, high soil populations of the lesion nematode *Pratylenchus neglectus* at planting were associated with reductions in grain yield of up to 71%. In this case, soil nematode populations of just 2000 per kilogram of soil were capable of limiting grain yield in intolerant varieties (Smiley *et al.*, 2005). Subsequent research demonstrated that nematicide application to control *P. neglectus* and *P. thornei* led to yield improvements of 31% and 18%, respectively, in spring cereals in the Pacific Northwest, and 9% and 11%, respectively, in winter cereals (Smiley, 2009). It was estimated that these two nematodes reduced wheat yield and profitability by as much as 5% in the Pacific Northwest states of Idaho, Oregon and Washington.

2.4 EFFECTS OF HERBIVORES ON GROWTH, DEVELOPMENT AND YIELD

Plants are a source of food for a great many species of invertebrate and vertebrate herbivores. Amazingly, however, in natural systems, most plants show little obvious damage, and indeed, although plants can be completely defoliated by herbivory, such occurrences are sporadic. The impact of herbivory on plant growth, development and reproductive output depends on several factors, (i) timing of the herbivory, (ii) location of the herbivory – what tissue is attacked and its age, (iii) the intensity of herbivory – how much plant tissue is consumed, and (iv) the frequency of herbivory – how often are the plants attacked (Crawley, 1997). In this section, we consider the effects of insect and vertebrate herbivores on plant performance. Determining the intensity of herbivory can be difficult, and as a result, estimates of plant losses to herbivory can differ greatly depending on the methods used (Schoonhoven *et al.*, 2005). This can make comparisons between different studies difficult. Nevertheless, the examples described in the following section serve to illustrate the extent of herbivory and provide a useful background to the physiological responses of plants covered in later chapters.

2.4.1 Effects of insect herbivores on plant growth, development and yield

Insects are estimated to consume approximately 10% of all plant biomass produced annually (Barbosa & Schultz, 1987; Coupe & Cahill, 2003). Precisely how much plant biomass is consumed will depend on a number of factors, such as the type of vegetation and the geographical location. Thus, tropical dry forests experience considerably greater herbivore pressure (14%) than temperate forests (7%) (Coley & Barone, 1996; Schoonhoven *et al.*, 2005). In tropical forests, there is a marked difference in patterns of herbivory, with daily rates of damage to young leaves up to 25 times greater than damage to mature leaves (Coley & Barone, 1996). Young leaves in tropical forests expand over a short period of 1–3 wk, and yet, herbivory

during this period accounts for 68% of the lifetime damage caused by herbivory. This compares to 27% of the lifetime damage from herbivory occurring on young leaves in temperate forests (Coley & Barone, 1996).

One approach to determining the impact of insect herbivory on plant growth is to remove insects using insecticides. This approach was used to examine the impact of insects on growth of eucalyptus trees. Spraying trees with insecticides not only reduced insect loads, but also led to substantial increases in tree growth. In two species of eucalyptus treated over several years with insecticide, the growth of main shoots was increased by between 100% and 380% (Fox & Morrow, 1992).

A further consideration is the mode of feeding of the insect. Damage caused by chewing insects is clearly visible, for example as holes in leaves. Such damage can exert a significant impact on plants both in the wild and under agronomic conditions. Chewing insects are responsible for 72% of the annual leaf consumption on Barro Colorado Island in Panama and are estimated to be responsible for 75% or more of the annual leaf consumption on the Parque Nacional Manu in Peru (Leigh, 1997). Herbivory by chewing insects can also exert a significant impact on crop plants. For example, grasshoppers are a major pest of crops worldwide and are responsible for an estimated annual crop loss of \$6 million on cereal crops in the United States (Gage & Mukerji, 1978). A grasshopper density of 75 per square metre on barley plants led to reductions of up to 47% in shoot biomass and 53% in root biomass, with reductions in grain yield of up to 36% (Fig. 2.5; Begna & Fielding, 2008). These reductions in growth and yield are substantial, and it should come as no surprise therefore that severe infestations can lead to crop losses of \$200 million in Canada and the United States (Gage & Mukerji, 1978).

Compared to chewing insects, it can be more difficult to determine plant losses resulting from feeding by sap-sucking insects. Intriguingly, phloem-feeding insects tend to be smaller than leaf-chewing insects but can consume more plant per gram of body mass (Coley & Barone, 1996). A study published in 1993 examined the impact of three species of insect herbivores, the xylem-sucking spittlebug (*Philaenus spumarius*), the phloem-sucking aphid (*Uroleucon caligatum*) and the leaf-chewing beetle (*Trirhabda* sp.), on goldenrod (*Solidago altissima*) (Meyer, 1993). The spittlebug was found to be the most damaging, while least damage was caused by the aphid. So although the beetle and the aphid reduced total leaf mass, total leaf area and root mass, the spittlebug caused five to six times more damage. According to Meyer (1993), the damage appeared to be the result of a reduction in leaf area per unit of leaf mass, rather than through any alterations in plant physiology.

Reproductive output can be markedly affected by even moderate levels of herbivory, and the magnitude of any reductions can depend on the types of insect herbivores. For example, defoliating insects were found to have little effect on acorn production by oaks (*Quercus robur*), whereas the removal of sucking insects by application of insecticides over several years led to increased acorn production (Crawley, 1985; Crawley, 1997).

2.4.2 Effects of vertebrate herbivores on plant growth, development and yield

Vertebrate herbivores can exert profound effects on plant growth and development and the structure and composition of plant communities. For example, in East African savannas, grazing by large ungulates increases primary production and alters the composition and structure of the vegetation (McNaughton, 1984). In contrast, arctic ecosystems are particularly sensitive to vertebrate grazing because of their low net primary productivity, and in this case, grazing

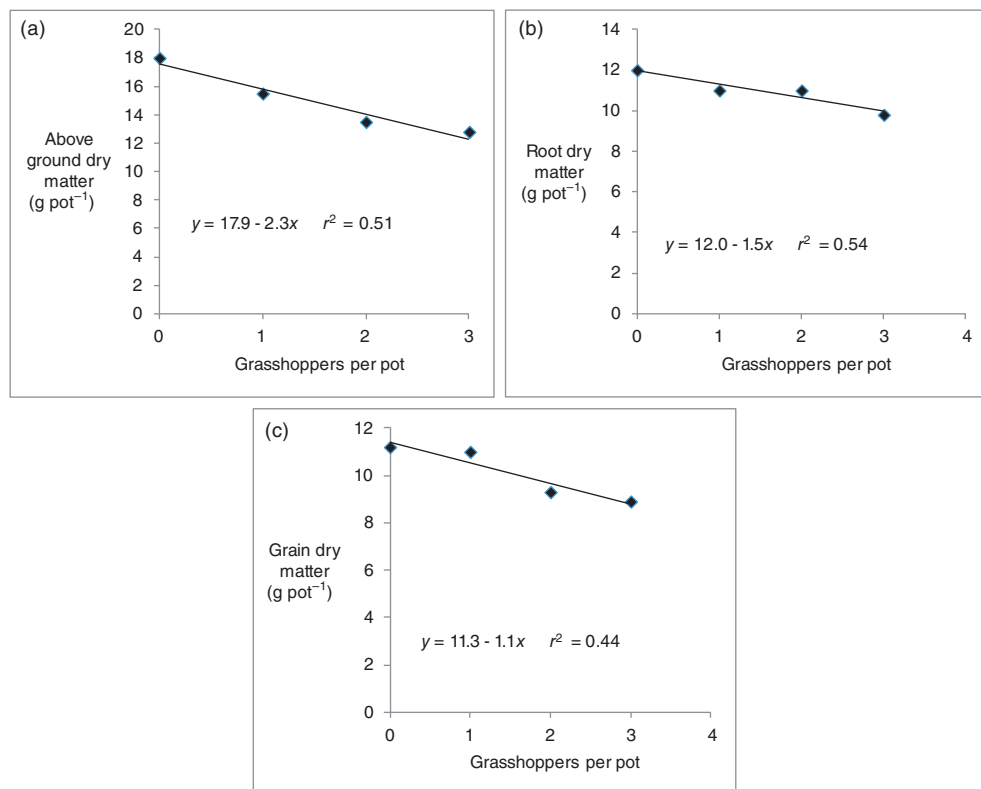


Fig. 2.5 Effects of grasshoppers on growth and yield of barley. Relationship between (a) above-ground dry matter (b) below-ground dry matter and (c) grain yield, and numbers of grasshoppers per pot. Above- and below-ground dry matter were determined at anthesis. Begna & Fielding (2008). Reproduced with permission from D. J. Fielding.

by vertebrates can decrease plant production (Batzli *et al.*, 1980). For example, snow geese in arctic Canada were shown to reduce above-ground biomass of two graminoids, *Eriophorum scheuchzeri* and *Dupontia fisheri*, consuming up to 113% and 78%, respectively, of the net above-ground primary production of the two plants (Fig. 2.6; Gauthier *et al.*, 1995).

For long-lived plants, the effect of a single herbivore species can vary markedly during the life of the plant. This was examined in a study of the effects of grazing by black-tailed deer (*Odocoileus hemionus columbianus*) and snails (*Helminthoglypta arrosa* and *Helix aspersa*) on the nitrogen-fixing shrub *Lupinus chamissonis* in a sand dune system in northern California (Warner & Cushman, 2002). Deer grazing significantly reduced the volume and growth rate of the lupins in the seedling and juvenile stages of development, but although grazing reduced shoot lengths in mature shrubs, there was no effect on growth rates. Furthermore, deer grazing of the mature shrubs increased inflorescence production but decreased seed mass. Interestingly, although snails were commonly found around the lupins, they had no significant effect on growth rate of the plants (Warner & Cushman, 2002).

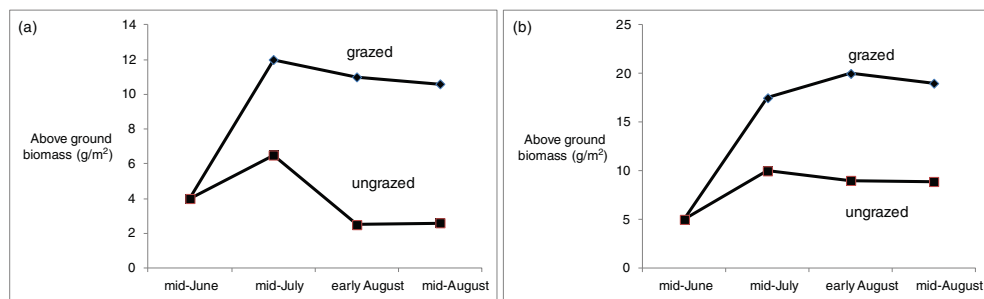


Fig. 2.6 Seasonal trends in above ground dry biomass of (a) *Eriophorum scheuchzeri* and (b) *Dupontia fisheri* in ungrazed areas and areas grazed by greater snow geese in 1993 on Bylot Island, NWT, Canada. Gauthier *et al.* (1995). Reproduced with permission of John Wiley & Sons.

Herbivory can induce alterations in the size and shape of plants, with consequences for plant competition and subsequent effects on other organisms (Danell & Bergström, 2002). Such alterations might occur as a result of removal of the leading shoot or apical bud of woody species. Indeed, the size and shape of plants can be altered even after removal of small amounts of biomass. Thus, although browsing of pine by moose during the summer results in just minor loss of plant biomass, growth of the leading shoot can be halted, resulting in a visible and long-lasting change in the architecture of the tree (Danell & Bergström, 2002). Vertebrate browsing can also lead to changes in the structure of plant communities. For example, browsing by moose on Isle Royale in Lake Superior, Michigan, prevented saplings of preferred species from growing into the tree canopy, resulting in a forest with fewer canopy trees and a well-developed understorey of shrubs and herbs (McInnes *et al.*, 1992).

More often than not, vertebrate herbivory does not result in plant death, either because most plants have some parts with low value for herbivores or because plants can compensate for damage (see Box 2.1). Nevertheless, mortality does occur, particularly when seedlings or young plants are damaged. Mortality can also occur among older, more mature plants. In mature trees, debarking is a major cause of mortality. Squirrels, rabbits and hares can kill large trees in their prime as a result of ring-barking and bark-stripping (Gill, 1992), while bark-stripping by voles can lead to mortality of both broadleaf and coniferous trees (Danell *et al.*, 1991; Hansson, 1994). Incredibly, as many as 96% of the mature trees in a *Terminalia glaucescens* woodland was recorded as being killed by elephant debarking (Laws *et al.*, 1975).

Box 2.1 Plants can compensate for damage caused by herbivory: lessons in tolerance

Although most plants are grazed or browsed by invertebrate and/or vertebrate herbivores, they are equipped with a variety of mechanisms that can reduce the damaging effects of herbivory. The capacity of plants to regrow after tissue loss can be regarded as tolerance, and the re-growth is reflected in final plant biomass (Augustine & McNaughton, 1998). The net effect of herbivory can be negative, positive or even zero, depending on a variety of factors, including availability of leaf area, meristems, stored nutrients, soil resources,

and the frequency and intensity of defoliation (Crawley, 1997). Importantly, the ability of plants to compensate for tissue loss as a result of herbivory depends on the timing of attack. In general, early attacks allow for the possibility of regrowth, while herbivory late in the season leaves little time for regrowth and might make grazed plants more vulnerable to harsh winter conditions.

The mechanisms that lead to compensatory regrowth after herbivore damage can be divided into intrinsic and extrinsic mechanisms (McNaughton, 1983). Intrinsic mechanisms involve changes in plant physiology and development and include increased photosynthetic rates in surviving leaves, redistribution of assimilate to production of new leaves and production of new shoots from dormant buds or newly produced buds. Extrinsic mechanisms involve modification of the environment and include increased light intensity for surviving leaf area, improved water and nutrient availability to the surviving leaf tissue and increased longevity of remaining leaves (McNaughton, 1983; Crawley, 1997).

In terms of the intrinsic mechanisms, increased rates of photosynthesis in remaining leaves might be the result of increased movement of cytokinins from roots to the fewer, remaining leaves, together with increased stomatal conductance in these leaves. Increased cytokinins can also lead to the activation of meristems, and in grasses, tillering is a well-known response to herbivory (McNaughton, 1983). Among the extrinsic mechanisms, loss of leaves as a result of herbivory will lead to less shading of lower leaves, thereby delaying senescence and prolonging longevity of the lower leaves.

In grasses, grazed tillers commonly have higher relative growth rates than ungrazed tillers, resulting in full compensation for tissue lost to defoliation (Crawley, 1997). However, full compensation might not occur if the defoliation is repeated, and moreover, such responses might be species dependent. Del-Val and Crawley (2005) carried out an assessment of defoliation tolerance in eight British grassland species, four herbivore increasers (species favoured under herbivory) and four herbivore decreasers (species not favoured under herbivory). They found that plant mortality increased with frequency and intensity of defoliation, and herbivore increaser species had significantly greater compensation ability than herbivore decreaser species (Fig. 2A). Most species were able to compensate for low levels of tissue loss, suggesting the existence of a threshold, below which herbivory would not be detrimental (Del-Val & Crawley, 2005). Indeed, such thresholds have been demonstrated for various plants. Thus, *Datura stramonium* can fully compensate for 10% defoliation, *Vaccinium myrtillus* for 50% defoliation and *Purshia tridentate* for 100% defoliation (Bilbrough & Richards, 1993; Tolvanen *et al.*, 1993; Fornoni & Nuñez-Farfán, 2000). In their study, Del-Val and Crawley (2005) found that the most critical stage for all species was the immature stage, when increased levels and frequency of defoliation led to disproportionately greater damage. This stage is probably very sensitive to defoliation because the plants are likely to have depleted their seed reserves, but are not yet fully established, and therefore unable to obtain all the nutrients and assimilates required for compensation.

Levels of herbivory are three times greater in aquatic systems than in terrestrial systems (Cyr & Pace, 1993). It stands to reason therefore that chemical defences should play a major role against herbivory in marine and freshwater macrophytes. Tolerance to herbivory can also be important but is dependent on the plant. Thus, tolerance to herbivory does not

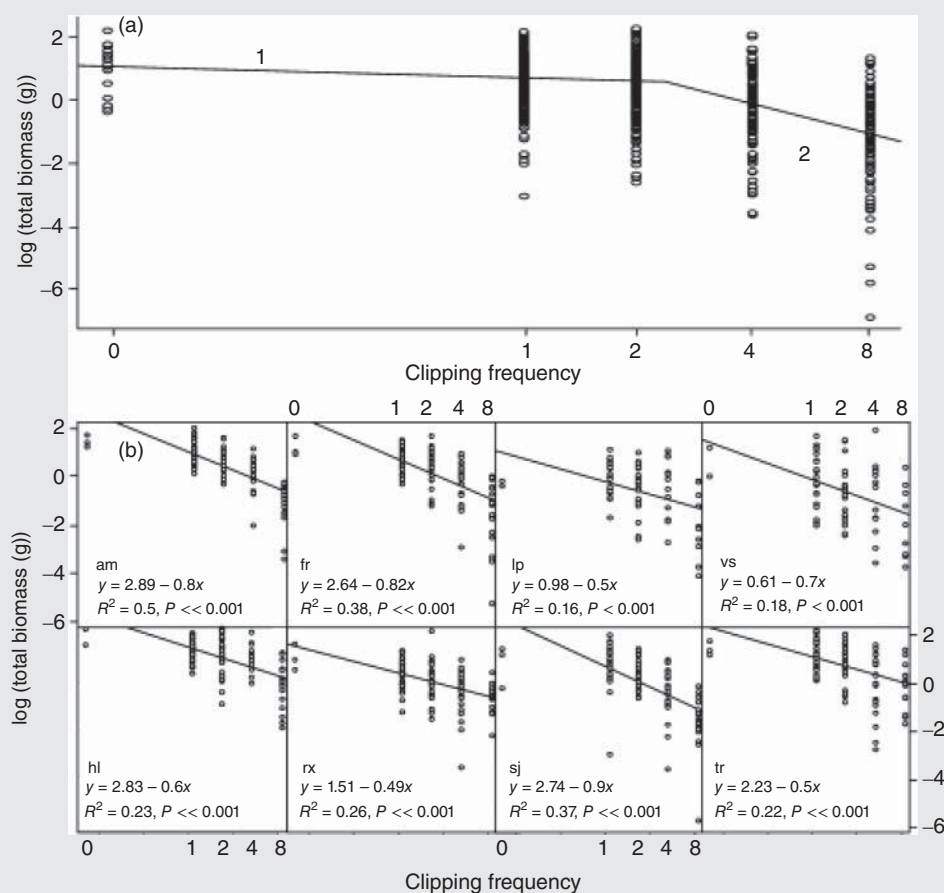


Fig. 2A Impact of clipping frequency (log scale) on total standing biomass of eight British grassland species. (a) Piecewise regression showing the existence of a threshold above more than two clippings. Points shown are final values of log(total standing biomass) across all timings and ontogenic stages. The two lines represent (1) minimal model for zero to twice clipped ($y = 0.76 - 0.04x$), (2) minimal model for greater than twice clipped ($y = 1.23 - 1.06x$). (b) Impact of clipping frequency per species. Upper panels show increaser species, and lower panels show decreasers. Note the species slope differences. Points represent final values of log(total standing biomass) and the lines represent linear regressions fitted to clipping frequency for each species. Species abbreviations are as follows: am, *Achillea millefolium*; fr, *Festuca rubra* ssp. *rubra*; hl, *Holcus lanatus*; lp, *Lathyrus pratensis*; rx, *Rumex acetosella*; sj, *Senecio jacobaea*; tr, *Trifolium repens*; vs, *Vicia sativa* ssp. *nigra*. Del-Val and Crawley (2005). Reproduced with permission of John Wiley & Sons.

appear to be common in algae, probably because of their simple morphology and functional organisation, lack of a root system for storing reserves and the presence of few, if any, lateral meristems that could be activated following damage to apical tissues. In contrast, marine vascular plants such as seagrasses possess many of the characteristics required for tolerance to herbivory in terrestrial plants, including the presence of largely inaccessible

basal meristems. In a study of the effects of simulated herbivory in the seagrass *Posidonia oceanica*, plants showed a significant ability to compensate for low and moderate levels of herbivory by increasing above-ground growth of damaged shoots (Vergés *et al.*, 2008). Interestingly, this increased shoot growth was not accompanied by increased photosynthesis. In addition, low levels of simulated herbivory did not affect stored resources, whereas nitrogen reserves appeared to be important in helping the plants compensate for damage under moderate and high levels of herbivory (Vergés *et al.*, 2008). Tolerance to herbivory was also demonstrated in the giant kelp *Macrocystis integrifolia*, which, similarly to most kelps, has specialised tissues for internal long-distance transport similar to that of higher plants, that is sieve tube elements (Raven, 2003). *M. integrifolia* exhibited compensatory growth following grazing by the amphipod *Peramphithoe femorata*, which was suggested to help the kelp tolerate moderate levels of grazing (Cerdeña *et al.*, 2009).

2.5 EFFECTS OF PARASITIC PLANTS ON GROWTH, DEVELOPMENT AND YIELD

As we saw in Chapter 1, parasitic angiosperms rely partially or totally on their host for supplies of organic and inorganic solutes and water. Generally, infection by parasitic plants reduces host productivity and/or reproductive effort, as reported for both root and shoot parasites (e.g. Matthies & Egli, 1999; Howell & Mathiasen, 2004). In some cases, such as heavy mistletoe infection, parasitism can lead to death of the host plant (Aukema, 2003).

A small number of genera infect crop plants and are capable of causing serious crop losses. For example, several species of *Striga* are important weeds in various parts of the semi-arid tropics. These are root hemiparasites, with *S. hermonthica* infecting grasses (e.g. maize, sorghum, millet and rice) and *S. gesnerioides* parasitic on various C₃ dicotyledenous hosts. Infection by *Striga* results in reduced biomass accumulation in host plants and can also alter allocation of biomass in the plant, resulting in substantial reductions in grain yield (Graves, 1995). In contrast to *Striga*, *Orobanch*e is an obligate root holoparasite, completely lacking in chlorophyll and as a result is dependent on its host for carbohydrate. Infection of tomato with *O. aegyptiaca* reduced host biomass even at low infection levels and decreased shoot : root ratio before any reduction in overall biomass accumulation (Fig. 2.7; Barker *et al.*, 1996). These effects increased with time, with significant reductions in host biomass being observed on emergence of the parasite shoots above the soil surface. Closer examination of biomass allocation revealed that leaf biomass was increased by infection, consistent with the increase in leaf area ratio in infected plants compared to the uninfected controls (Fig. 2.7). This was accompanied by a decreased unit leaf rate (i.e. the efficiency of photosynthetic tissue in producing new leaves), suggesting that the overall carbon gain per unit of leaf area or weight was lower, as a result of either reduced rates of photosynthesis, increased rates of respiration, or both (Fig. 2.7; Barker *et al.*, 1996).

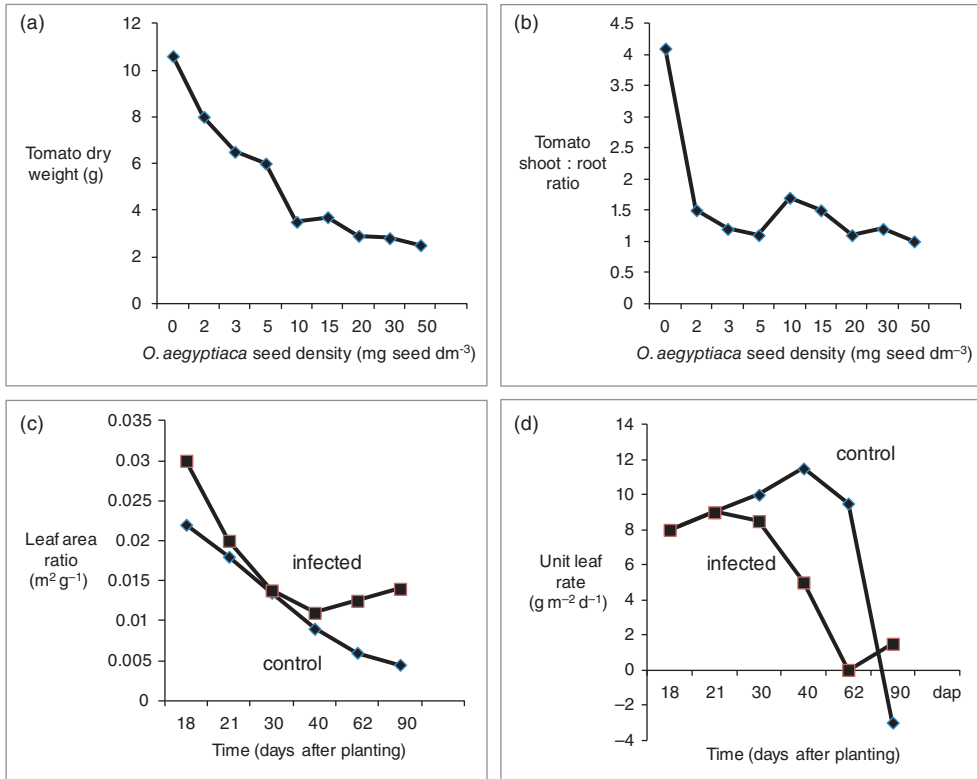


Fig. 2.7 Effect of the parasitic plant *Orobancha aegyptiaca* on growth of tomato. Effect of seed density of *O. aegyptiaca* on (a) total plant dry weight and (b) shoot : root ratio. The relationship between (c) leaf area ratio and (d) unit leaf rate in tomato plants in the absence or presence of *O. aegyptiaca*. Barker *et al.* (1996). Reproduced with permission of John Wiley & Sons.

2.6 CONCLUSIONS

The examples described in this chapter illustrate that parasitism and herbivory can have serious consequences for host plants, reducing growth and reproductive output and, at worst, leading to plant death. Parasitism and herbivory can lead to changes in competitive balances between host and non-host species and as a result can affect community structure and population dynamics. For crop plants, parasitism and herbivory can lead to catastrophic crop losses, some of which have had a profound influence on humans. The potato late blight epidemics of the mid-1800s are just one example, and the influence of parasites and herbivores continues relentlessly.

In this chapter, we have considered the effects of parasitism and herbivory on plant and crop growth, development and yield. The mechanisms underlying these changes have only been hinted at but are dealt with in more detail in the subsequent chapters.

RECOMMENDED READING

- Oerke EC, 2006. Crop losses to pests. *Journal of Agricultural Science* **144**, 31–43.
- Schumann GL, D'Arcy CJ, 2012. *Hungry planet: stories of plant diseases*. St Paul, Minnesota: APS Press.
- Spallek T, Mutuku M, Shirasu K, 2013. The genus *Striga*: a witch profile. *Molecular Plant Pathology* **14**, 861–869.

REFERENCES

- Arnitzen FK, Visser JHM, Hoogendoorn J, 1994. The effect of the potato cyst nematode *Globodera pallida* on *in vitro* root growth of potato genotypes differing in tolerance. *Annals of Applied Biology* **124**, 59–64.
- Augustine DJ, McNaughton SJ, 1998. Ungulate effects on the functional species composition of plant communities: herbivore selectivity and plant tolerance. *Journal of Wildlife Management* **62**, 1165–1183.
- Aukema JE, 2003. Vectors, viscin, and Viscaceae: mistletoes as parasites, mutualists, and resources. *Frontiers in Ecology and the Environment* **1**, 212–219.
- Barbosa P, Schultz JC, 1987. *Insect outbreaks*. San Diego: Academic Press.
- Barker ER, Press MC, Scholes JD, Quick WP, 1996. Interactions between the parasitic angiosperm *Orobanchae aegyptiaca* and its tomato host: growth and biomass allocation. *New Phytologist* **133**, 637–642.
- Batzli GO, White RG, MacLean SF, Pitelka FA, Collier BD, 1980. The herbivore-based trophic system. In: Brown J, Miller PC, Tieszen LL, Bunnell FL, eds. *An arctic ecosystem: the coastal tundra at Barrow Alaska*. Stroudsburg, PA: Dowden, Hutchinson & Ross, Inc., pp. 335–410.
- Begna SH, Fielding DJ, 2008. Growth and yield of barley in relation to grasshopper feeding damage. *Canadian Journal of Plant Science* **88**, 219–217.
- Bilbrough CJ, Richards JH, 1993. Growth of sagebush and bitterbrush following simulated winter browsing: mechanisms of tolerance. *Ecology* **74**, 481–492.
- Burnett PA, Mezzalama M, 1990. The barley yellow dwarf screening programme at CIMMYT. In: Burnett PA, ed. *World perspectives on barley yellow dwarf*. Mexico: CIMMYT, pp. 434–440.
- Carver TLW, Griffiths E, 1981. Relationship between powdery mildew infection, green leaf area and grain yield of barley. *Annals of Applied Biology* **99**, 255–266.
- Cerda O, Karsten U, Rothausler E, Tala F, Thiel M, 2009. Compensatory growth of the kelp *Macrocystis integrifolia* (Phaeophyceae, Laminariales) against grazing of *Peramphithoe femorata* (Amphipoda, Ampithoidae) in northern-central Chile. *Journal of Experimental Marine Biology and Ecology* **377**, 61–67.
- Coley PD, Barone JA, 1996. Herbivory and plant defences in tropical forests. *Annual Review of Ecology and Systematics* **27**, 305–335.
- Coupe MD, Cahill JF, 2003. Effects of insects on primary production in temperate herbaceous communities: a meta-analysis. *Ecological Entomology* **28**, 511–521.
- Crawley MJ, 1985. Reduction of oak fecundity by low density herbivore populations. *Nature* **314**, 163–164.
- Crawley MJ, 1997. Plant-herbivore dynamics. In: Crawley MJ, ed. *Plant ecology*. Oxford: Blackwell Publishing, pp. 401–474.
- Cyr H, Pace ML, 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* **361**, 148–150.
- Danell K, Bergström R, 2002. Mammalian herbivory in terrestrial environments. In: Herrera CM, Pellmyr O, eds. *Plant-Animal Interactions: An Evolutionary Approach*. Oxford: Blackwell Publishing Ltd., pp. 107–131.
- Danell K, Hjalten J, Ericson L, Elmqvist M, 1991. Vole feeding on male and female willow shoots along a gradient of plant productivity. *Oikos* **62**, 145–152.
- Del-Val E, Crawley MJ, 2005. Are grazing increaser species better tolerators than decreasers? An experimental assessment of defoliation tolerance in eight British grassland species. *Journal of Ecology* **93**, 1005–1016.
- Doodson JK, Manners JG, Myers A, 1964. Some effects of yellow rust (*Puccinia striiformis*) on the growth and yield of a spring wheat. *Annals of Botany* **28**, 459–472.
- Edwards MC, Fetch TG, Schwarz PB, Steffenson BJ, 2001. Effect of barley yellow dwarf virus infection on yield and malting quality of barley. *Plant Disease* **85**, 202–207.
- Fornoni J, Nuñez-Farfán J, 2000. Evolutionary ecology of *Datura stramonium*: Genetic variation and costs for tolerance to defoliation. *Evolution* **54**, 789–797.

- Fox LR, Morrow PA, 1992. Eucalyptus responses to fertilization and reduced herbivory. *Oecologia* **89**, 214–222.
- Gage SH, Mukerji MK, 1978. Crop losses associated with grasshoppers in relation to economics of crop production. *Journal of Economic Entomology* **71**, 487–498.
- Garry G, Jeuffroy MH, Tivoli B, 1998. Effects of Aschochyta blight (*Mycosphaerella pinodes* Berk. & Blox.) on biomass production, seed number and seed weight of dried pea (*Pisum sativum*) as affected by plant growth stages and disease intensity. *Annals of Applied Biology* **132**, 49–59.
- Gauthier G, Hughes RJ, Reed A, Beaulieu J, Rochefort L, 1995. Effect of grazing by greater snow geese on the production of graminoids at an arctic site (Bylot Island, NWT, Canada). *Journal of Ecology* **83**, 653–664.
- Gill RMA, 1992. A review of damage by mammals in north temperate forests. 3. Impact on trees and forests. *Forestry* **65**, 363–388.
- Graves JD, 1995. Host plant responses to parasitism. In: Press MC, Graves JD, eds. *Parasitic Plants*. London: Chapman and Hall., pp. 206–225.
- Halila MH, Strange RN, 1996. Identification of the causal agent of wilt of chickpea in Tunisia as *Fusarium oxysporum* f.sp. *ciceri* race 0. *Phytopathologia Mediterranea* **35**, 67–74.
- Hansson L, 1994. Bark consumption by voles in relation to geographical origin of tree species. *Scandinavian Journal of Forest Research* **9**, 288–296.
- Howell BE, Mathiasen RL, 2004. Growth impacts of *Psittacanthus angustifolius* Kuijt on *Pinus oocarpa* Schiede in Honduras. *Forest Ecology and Management* **198**, 75–88.
- James WC, 1967. Assessment of barley leaf blotch and its effect on spring barley yield. (Proceedings of the 1967 British Insecticide and Fungicide Conference), 111–114.
- de Jesus Junior WC, do Vale FXR, Coelho RR, Hau B, Zambolim L, Costa C, Bergamin Filho A, 2001. Effects of angular leaf spot and rust on yield loss in *Phaseolus vulgaris*. *Phytopathology* **91**, 1045–1053.
- Jordan VWL, Best GR, Allen EC, 1985. Effects of *Pyrenophora teres* on dry matter production and yield components of winter barley. *Plant Pathology* **34**, 200–206.
- Kalorizou HA, Gowen SR, Wheeler TR, 2007a. Genotypic differences in the growth of bananas (*Musa* spp.) infected with migratory endoparasitic nematodes. I. Roots. *Experimental Agriculture* **43**, 331–342.
- Kalorizou HA, Gowen SR, Wheeler TR, 2007b. Genotypic differences in the growth of bananas (*Musa* spp.) infected with migratory endoparasitic nematodes. II. Shoots. *Experimental Agriculture* **43**, 343–352.
- Kuribayashi K, Shinkai A, 1952. On the new disease of rice, black-streaked dwarf. *Annals of the Phytopathological Society of Japan* **16**, 41.
- Large EC, Doling DA, 1962. The measurement of cereal mildew and its effect on yield. *Plant Pathology* **11**, 47–57.
- Last FT, 1962. Analysis of the effects of *Erysiphe graminis* D.C. on the growth of barley. *Annals of Botany* **26**, 279–289.
- Laws RM, Parker ISC, Johnstone RCB, 1975. *Elephants and their habitats. The ecology of elephants in north Bunyoro, Uganda*. Oxford: Clarendon Press.
- Leigh EG Jr, 1997. *Ecology of tropical forests: the view from Barro Colorado*. New York: Oxford University Press.
- Matthies D, Egli P, 1999. Response of a root hemiparasite to elevated CO₂ depends on host type and soil nutrients. *Oecologia* **120**, 156–161.
- McInnes PF, Naiman RJ, Pastor J, Cohen Y, 1992. Effects of moose browsing on vegetation and litter of the boreal forest, Isle Royal, Michigan, USA. *Ecology* **73**, 2059–2075.
- McNaughton SJ, 1983. Compensatory growth as a response to herbivory. *Oikos* **40**, 329–336.
- McNaughton SJ, 1984. Grazing lawns: animals in herds, plant form and coevolution. *American Naturalist* **124**, 863–886.
- Meyer GA, 1993. A comparison of the impacts of leaf feeding and sap feeding insects on growth and allocation of goldenrod. *Ecology* **74**, 1101–1116.
- Navas-Cortéz JA, Alcalá-Jiménez AR, Hau B, Jiménez-Díaz RM, 2000. Influence of inoculum density of races 0 and 5 of *Fusarium oxysporum* f.sp. *ciceris* on development of Fusarium wilt in chickpea cultivars. *European Journal of Plant Pathology* **106**, 135–146.
- Paul ND, Ayres PG, 1986a. The impact of a pathogen (*Puccinia lagenophorae*) on populations of groundsel (*Senecio vulgaris*) overwintering in the field. I. Mortality, vegetative growth and the development of size hierarchies. *Journal of Ecology* **74**, 1069–1084.
- Paul ND, Ayres PG, 1986b. The impact of a pathogen (*Puccinia lagenophorae*) on populations of groundsel (*Senecio vulgaris*) overwintering in the field II. Reproduction. *Journal of Ecology* **74**, 1085–1094.

- Paul ND, Ayres PG, 1987. Survival, growth and reproduction of groundsel (*Senecio vulgaris*) infected by rust (*Puccinia lagenophorae*) in the field during summer. *Journal of Ecology* **75**, 61–71.
- Pegg GF, Dixon GR, 1969. The reactions of susceptible and resistant tomato cultivars to strains of *Verticillium albo-atrum*. *Annals of Applied Biology* **63**, 389–400.
- Raven JA, 2003. Long-distance transport in non-vascular plants. *Plant Cell and Environment* **26**, 73–85.
- Ruan YL, Chen SX, Lin RF, Jiang WL, Jin DD, 1984. Studies on rice black-streaked dwarf disease. *Zhejiang Agricultural Sciences* **1984**, 185–187.
- Sadras VO, Quiroz F, Echarte L, Escande A, Pereyra VR, 2000. Effect of *Verticillium dahlia* on photosynthesis, leaf expansion and senescence of field grown sunflower. *Annals of Botany* **86**, 1007–1015.
- Schoonhoven LM, van Loon JJA, Dicke M, 2005. *Insect-plant biology*. Oxford: Oxford University Press.
- Scott SW, Griffiths E, 1980. Effects of controlled epidemics of powdery mildew on grain yield of spring barley. *Annals of Applied Biology* **94**, 19–31.
- Smiley RM, 2009. Root lesion nematodes reduce yield of intolerant wheat and barley. *Agronomy Journal* **101**, 1322–1335.
- Smiley RM, Whittaker RG, Gourlie JA, Easley SA, 2005. Suppression of wheat growth and yield by *Pratylenchus neglectus* in the Pacific Northwest. *Plant Disease* **89**, 958–968.
- Su H, Hwang SF, Chang KF, Conner RL, Xue AG, Warkentin TD, Blade SF, Turnbull GD, 2006. Assessment of yield loss caused by *Mycosphaerella* blight in pea crops in Western Canada. *Journal of Plant Protection and Diseases* **113**, 267–274.
- Trapero-Casas A, Jiménez-Díaz RM, 1985. Fungal wilt and root rot diseases of chickpea in southern Spain. *Phytopathology* **75**, 1146–1151.
- Tolvanen A, Laine K, Pakonen T, Saari E, Havas P, 1993. Aboveground growth responses of the bilberry (*Vaccinium myrtillus* L.) to simulated herbivory. *Flora (Jena)* **188**, 197–202.
- Vergés A, Pérez M, Alcoverro T, Romero J, 2008. Compensation and resistance to herbivory in seagrass: induced responses and simulated consumption by fish. *Oecologia* **155**, 751–760.
- Walters DR, Ayres PG, 1981. Growth and branching pattern of roots of powdery mildew infected barley. *Annals of Botany* **47**, 159–163.
- Wang H-D, Chen J-P, Wang A-G, Jiang X-H, Adams MJ, 2009. Studies on the epidemiology and yield losses from rice black-streaked dwarf disease in a recent epidemic in Zhejiang province, China. *Plant Pathology* **58**, 815–825.
- Warner PJ, Cushman JH, 2002. Influence of herbivores on a perennial plant: variation with life history stage and herbivore species. *Oecologia* **132**, 77–85.
- Xue AG, Warkentin TD, 2001. Partial resistance to *Mycosphaerella pinodes* in field pea. *Canadian Journal of Plant Science* **81**, 535–540.

3 **Photosynthesis in Attacked Plants and Crops**

3.1 **INTRODUCTION**

In the previous chapter, we saw that attack by pathogens, herbivores or parasitic plants can lead to substantial reductions in plant growth and reproductive output. This, in turn, can result in serious crop losses in crop production systems and altered community structure in natural systems. The mechanisms underlying altered plant growth, development and yield can vary depending on the plant and its attacker, including the plant organ attacked, and whether the attacker is a biotroph or necrotroph, whether it is a root or shoot parasite and whether it is a chewing insect or a sapsucker. Attacked plants can lose leaf area and root surface, or more insidiously, their vascular tissues can be disrupted. It is important to remember, however, that quite often, only part of the plant is attacked, and when considering plant responses, both attacked and non-attacked tissues need to be examined. Furthermore, as discussed in Chapter 2, plants can compensate for damage.

Rates of photosynthesis can be altered considerably after attack, although the nature of the change (i.e. decreased or increased photosynthetic rates) and the underlying mechanisms will depend on the plant–attacker interaction. When writing this chapter, I have assumed that the readers will have an understanding of photosynthesis. If some re-familiarisation with this process is necessary, I recommend the excellent accounts provided by Smith *et al.* (2010) and Scott (2008).

3.2 **PHOTOSYNTHESIS IN DISEASED PLANTS**

A common response to pathogen infection is a reduction in the rate of photosynthesis in the infected leaves. How this change is brought about can vary, depending, for example, on whether the pathogen is a biotroph or necrotroph, whether it is a virus or a bacterium, whether it produces a toxin and also which tissues are attacked. As indicated previously, another important consideration is the heterogeneity of infection, which can apply not only to the whole plant, but also to single leaves.

3.2.1 Photosynthesis in plants infected with biotrophic fungal pathogens

3.2.1.1 Changes in whole leaves

Infection by biotrophic fungal pathogens, such as downy mildews, powdery mildews and rusts, commonly leads to reduced rates of net photosynthesis (Fig. 3.1). For example, So & Thrower (1976) examined the effects of light and heavy infection with the rust fungus, *Uromyces appendiculatus*, on rates of photosynthesis in the second trifoliolate leaves of the legume *Vigna sesquipedalis*. In the lightly infected plants, rates of photosynthesis were reduced by 14% 7 days after inoculation, and by 17 days after inoculation, photosynthetic rates were reduced by 45% compared to uninfected leaves (Fig. 3.1a; So & Thrower, 1976). In contrast, photosynthesis was reduced earlier, more rapidly, and more substantially, in heavily infected plants compared to uninfected plants. Thus, rates of photosynthesis in heavily infected leaves were reduced by 18% by 4 days after inoculation, while by 17 days after inoculation, photosynthesis in infected leaves was reduced by 73% compared to the uninfected controls (Fig. 3.1a; So & Thrower, 1976). Similar steady and substantial reductions in rates of photosynthesis were observed in oak leaves infected with the powdery mildew fungus, *Microsphaera alphitoides* (Hewitt & Ayres, 1975; Fig. 3.1b) and *Arabidopsis thaliana* infected with the white

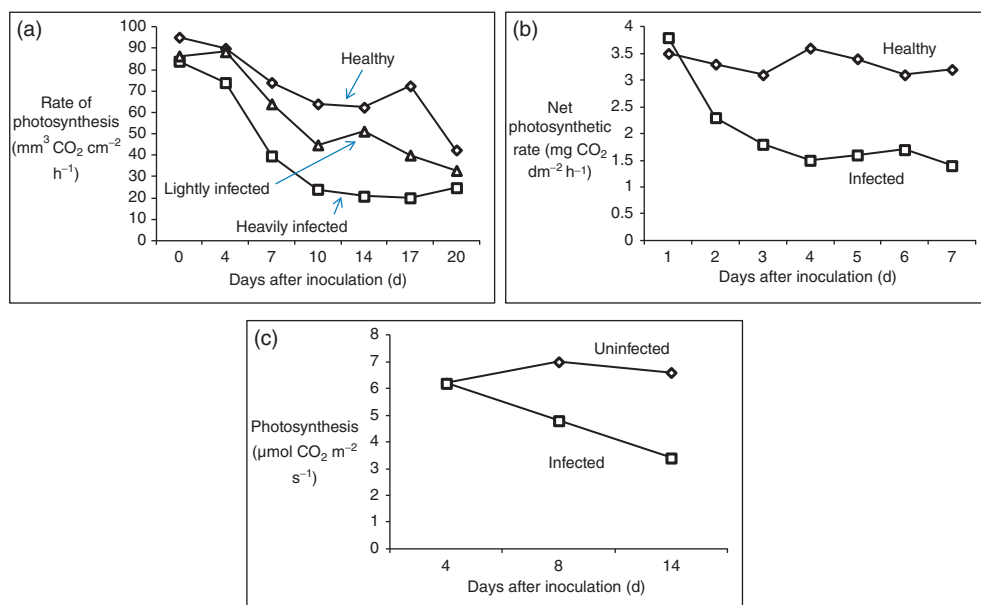


Fig. 3.1 Photosynthesis in leaves infected with biotrophic fungal pathogens. (a) effects of the rust, *Uromyces appendiculatus*, on photosynthesis in the second trifoliolate leaves of *Vigna sesquipedalis*. Leaves were either not infected (Healthy), lightly infected or heavily infected. Data from So and Thrower (1976). (b) effects of infection by the powdery mildew fungus, *Microsphaera alphitoides*, on photosynthetic rates in oak leaves. Hewitt and Ayres (1975). Reproduced with permission of Elsevier. (c) Rates of photosynthesis in leaves of *Arabidopsis thaliana* infected with the white blister rust pathogen, *Albugo candida*. Tang *et al.* (2006). Reproduced with permission of John Wiley & Sons.

blister rust, *Albugo candida* (Fig. 3.1c; Tang *et al.*, 1996). However, steady and substantial reductions in rates of net photosynthesis are not always observed, and indeed, exactly what happens to photosynthesis is dependent not only on the host–pathogen interaction, but also on the host plant variety. This is nicely illustrated by the work of Haigh *et al.* (1991), who examined changes in rates of net photosynthesis in four genotypes of oat (*Avena sativa*) differing in partial resistance to the powdery mildew fungus, *Erysiphe graminis* f.sp. *avenae*. Powdery mildew infection reduced rates of net photosynthesis in second leaves of the susceptible genotype Mostyn, with significant reductions evident from 5 days after inoculation (Fig. 3.2; Haigh *et al.*, 1991). In a more susceptible genotype (1674), no such reduction was observed, while in the resistant genotypes Maldwyn and 1621, rates of net photosynthesis were reduced, but only later in the experiment, 9 days after inoculation (Fig. 3.2). Interestingly, powdery mildew had no effect on photosynthesis in fifth leaves on these plants. Thus, the decline in photosynthesis, which is usually associated with infection with powdery mildews, appeared to be delayed or compensated for in oat (Haigh *et al.*, 1991).

Reductions in rates of photosynthesis could result from alterations in one or more of several mechanisms, including altered stomatal behaviour, decreased chlorophyll concentrations, perturbations in the light reactions of photosynthesis and reductions in the activities and amounts of Calvin cycle enzymes. Powdery mildew and rust infections can lead to altered stomatal behaviour (see Chapter 6), and such changes can influence rates of photosynthesis. For example, in pea plants infected with the powdery mildew fungus *Erysiphe pisi*, stomata opened more widely in the light in infected leaves than in healthy leaves 24 hours after inoculation. Thereafter, stomatal opening was progressively reduced by infection, and stomata failed to close completely in the dark until, 7 days after inoculation, all movements ceased and stomata remained partly open (Ayres, 1976). In this case, reduced stomatal opening was thought to be partly responsible for reduced rates of net photosynthesis during the later stages of infection (Ayres, 1976).

In leaves infected with biotrophic fungal pathogens, chlorosis is a common symptom, and indeed, there is often a progressive decline in chlorophyll content in such leaves. Scholes and Farrar (1987) observed a steady decline in total chlorophyll in barley leaves infected with brown rust, while the decline in chlorophyll concentration in leaves of *A. thaliana* infected with *A. candida* correlated well with the decline in photosynthetic rate in infected leaves (Tang *et al.*, 1996) (Fig. 3.3). A significant correlation between reduced rates of photosynthesis and chlorophyll levels was also found in leaves of *V. sesquipedalis* infected with rust (So & Thrower, 1976). However, such correlations are not always observed, as demonstrated in wheat infected with the rust *Puccinia striiformis* (Doodson *et al.*, 1964). Indeed, in oak infected with powdery mildew, photosynthesis started to decline before total chlorophyll levels (Hewitt, 1976).

What about changes in the light reactions of photosynthesis? After all, chloroplasts in leaves infected with rusts and powdery mildews undergo marked changes in ultrastructure, particularly in the later stages of infection. Powdery mildew infection of sugar beet and rust infection of broad bean were found to effect a preferential inhibition of non-cyclic photophosphorylation in isolated chloroplasts (Montalbini & Buchanan, 1974; Magyarosy *et al.*, 1976). Chloroplasts isolated from infected leaves showed a substantial (~45%) decrease in the rate of non-cyclic electron transport (water as the electron donor and NADP or ferricyanide as the electron acceptor) and attendant phosphorylation. Infection had no effect on the coupling of phosphorylation to photosynthetic electron transport (photophosphorylation), as determined

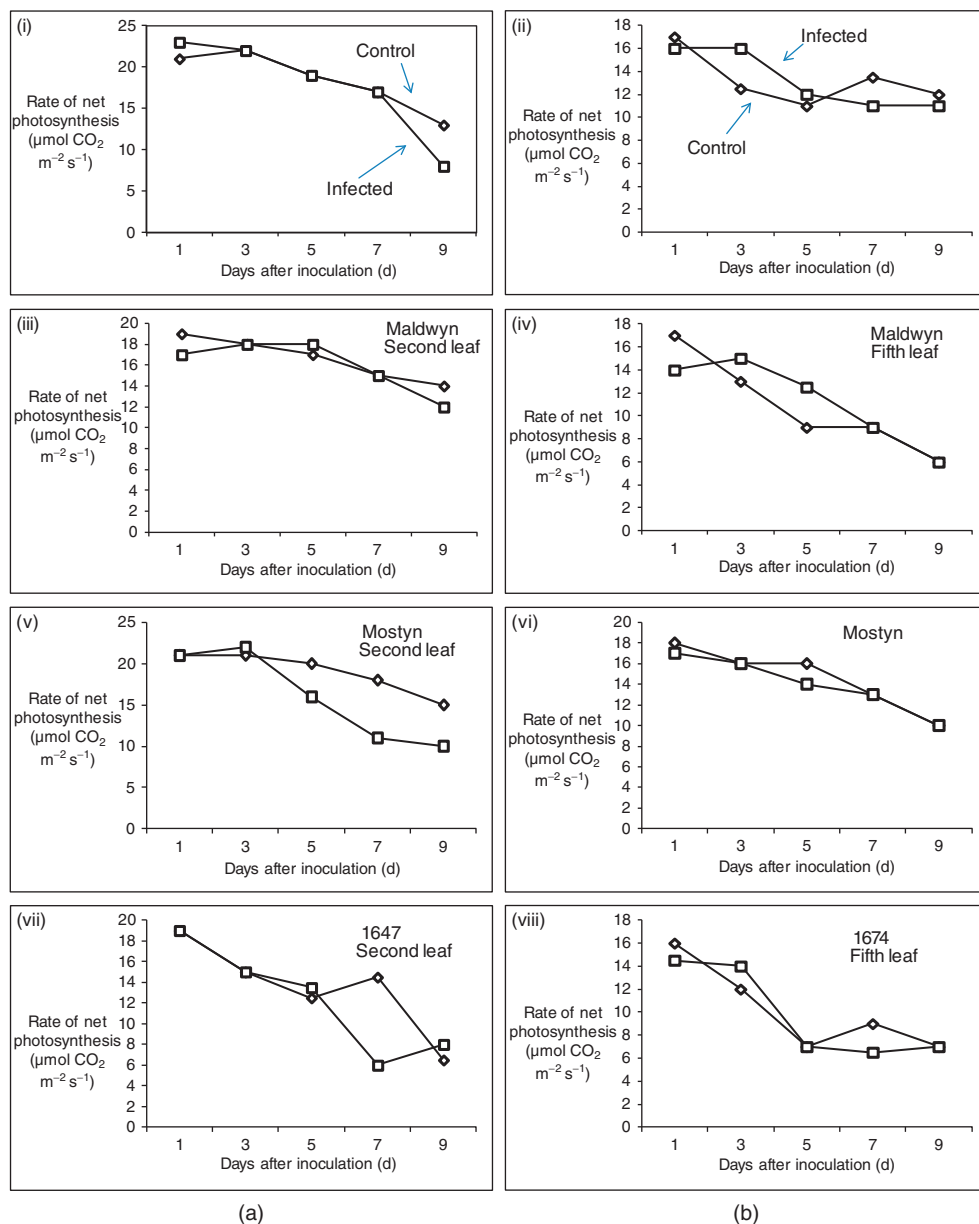


Fig. 3.2 Changes in the rate of net photosynthesis in infected and control oat leaves, exhibiting different levels of partial resistance to powdery mildew. Second leaf (a) and fifth leaf (b). Haigh *et al.* (1991). Reproduced with permission of John Wiley & Sons.

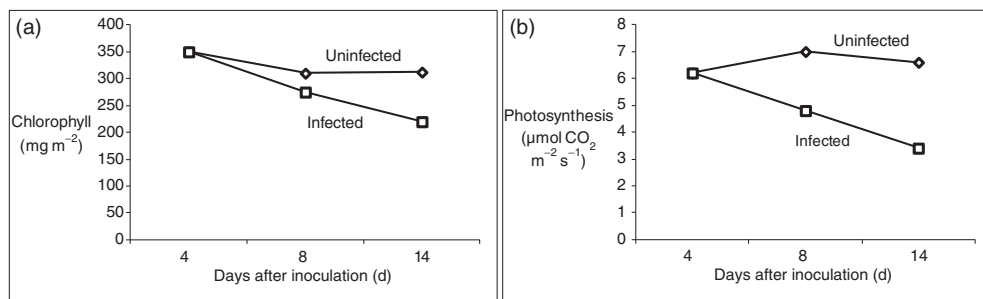


Fig. 3.3 Chlorophyll concentration (a) and rates of net photosynthesis (b) in leaves of *Arabidopsis thaliana* infected with the white blister rust pathogen, *Albugo candida*. Tang *et al.* (2006). Reproduced with permission of John Wiley & Sons.

by the ratio of ATP formed to NADP reduced (P:2e) (Magyarosy *et al.*, 1976). This suggests that the pathogen causes a block in the non-cyclic electron transport chain. Initial thoughts that this effect might have been the result of a pathogen-produced compound were dispelled when extensive washing of chloroplast membranes from rust-infected leaves produced no relief from the pathogen-induced reduction in the activity of the non-cyclic electron transport chain (Montalbini *et al.*, 1981). In fact, it is more likely that infection leads to alterations in the components of the non-cyclic electron transport chain, because the cytochrome content of the electron transport chain was decreased by approximately 33% in chloroplasts isolated from sugar beet leaves infected with powdery mildew (Magyarosy & Malkin, 1978). Because the photosystem I (PS I) and photosystem II (PS II) reaction centres and the bound iron-sulphur proteins were unaffected by infection, it appears that infection by these obligately biotrophic pathogens specifically altered the content of certain carriers involved in the electron transport chain, thereby reducing the rate of non-cyclic electron transport. Later work using chlorophyll fluorescence kinetics demonstrated a progressive decline in the rate of photosynthetic electron transfer in leaves of a susceptible barley variety infected with the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei* (Swarbrick *et al.*, 2006).

Substantial changes in the activities of Calvin cycle enzymes have also been found to occur in leaves infected with biotrophic fungal pathogens. Infection of barley leaves with the powdery mildew fungus led to a significant reduction in activity of the CO_2 fixing

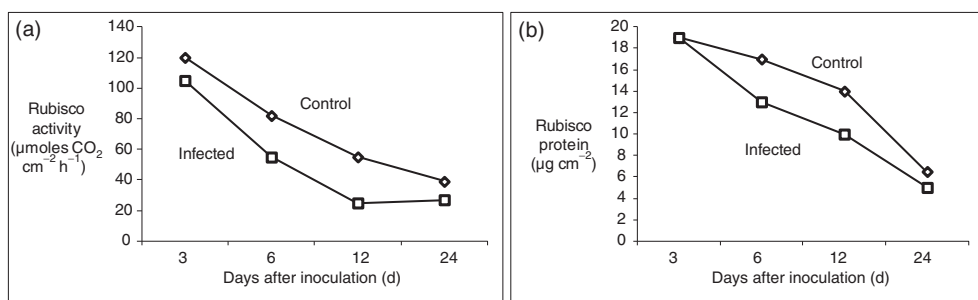


Fig. 3.4 Effects of powdery mildew infection on (a) Rubisco activity and (b) Rubisco protein in barley leaves. Walters and Ayres (1984). Reproduced with permission of John Wiley & Sons.

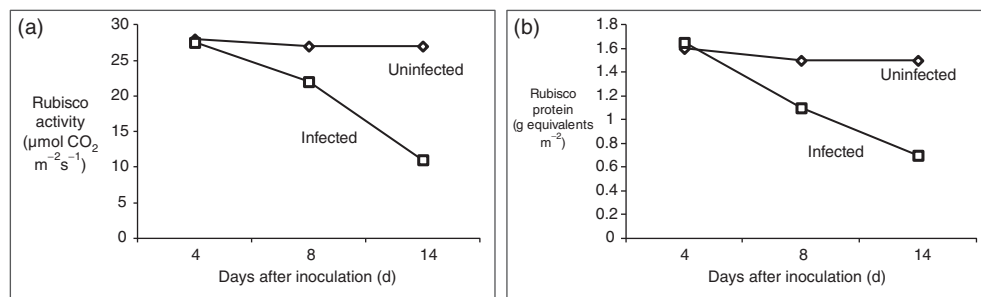


Fig. 3.5 Rubisco activity (a) and Rubisco protein (b) in leaves of *Arabidopsis thaliana* infected with the white blister rust pathogen, *Albugo candida*. Tang *et al.* (2006). Reproduced with permission of John Wiley & Sons.

enzyme, ribulose-1,5-bisphosphate carboxylase (Rubisco), brought about by a reduction in the amount of Rubisco protein after infection (Fig. 3.4; Walters & Ayres, 1984). A similar situation was observed in *A. thaliana* infected with the white blister rust *A. candida*, with reductions in Rubisco protein and activity of about 50% by 2 weeks after inoculation (Fig. 3.5; Tang *et al.*, 1996). Interestingly, work on powdery-mildew-infected sugar beet leaves found a decrease in the amount of Rubisco protein, but not its specific activity (activity per unit protein) after infection (Gordon & Duniway, 1982). However, as the latter authors pointed out, Rubisco activity might not be responsible for limiting the flux of carbon through the Calvin cycle, as the activities of other enzymes in that pathway might also be affected by infection. This was examined in barley leaves infected with powdery mildew, and activities of three enzymes of the pathway (3-Phosphoglycerate kinase, NAD⁺ glyceraldehyde-3-phosphate dehydrogenase and NADP⁺ glyceraldehyde-3-phosphate dehydrogenase) were found to be reduced substantially after infection, with possible implications for the regeneration of ribulose-1,5-bisphosphate (Walters & Ayres, 1984).

Perhaps, reductions in Rubisco protein and activity in leaves infected with biotrophic fungal pathogens should not be surprising, as it was well established that powdery mildew infection reduced ribosomes and rRNA in chloroplasts (e.g. Bennett & Scott, 1971; Dyer & Scott, 1972). In fact, subsequent work showed that mRNA coding for the small and large subunits of Rubisco was reduced substantially in barley leaves infected with powdery mildew, with reductions already evident just 1 day after inoculation (Higgins *et al.*, 1985). This was confirmed by later work, which showed that the expression of genes encoding Rubisco and chlorophyll a/b-binding protein was reduced substantially in a compatible interaction between barley and powdery mildew (Swarbrick *et al.*, 2006).

3.2.1.2 Changes in localised regions of infected leaves

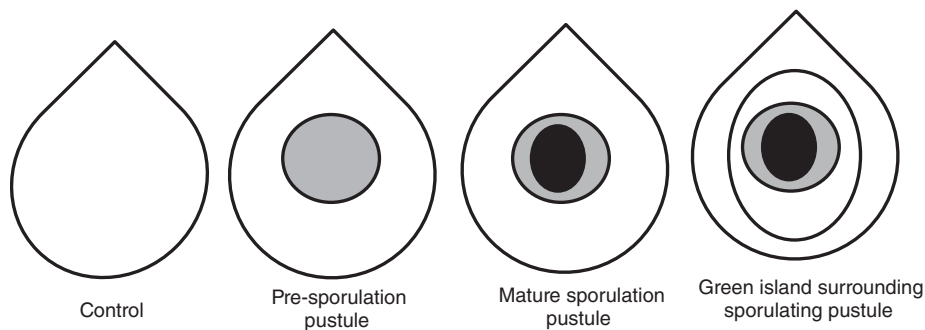
From the previous section, it is clear that infection by biotrophic fungal pathogens such as powdery mildews and rusts can reduce rates of net photosynthesis in whole leaves. What is less clear, perhaps, is the relative importance of individual partial processes in accounting for reduced photosynthetic rates in different host–pathogen interactions. In leaves of many host plants, infection by a rust or powdery mildew does not lead to uniform coverage of the leaf surface. Indeed, fungal pustules can be separated by areas of the leaf that appear to be uninfected. Is it possible that photosynthesis and its partial processes might be differentially

affected in these different regions of the leaf? Well, a sizeable body of work over the past 25 years or so shows that different regions of an infected leaf can behave quite differently.

As hinted previously, leaves infected with biotrophic fungal pathogens are often heterogeneous, consisting of cells invaded by the fungus, as well as cells that are not invaded but nevertheless are modified by the presence of the pathogen. In addition, although the area of leaf surrounding a rust pustule might appear, at least to the naked eye, to be free of fungal growth, there will be hyphae radiating out from the pustule into the surrounding mesophyll tissue. There will also be changes within the pustule, as tissues at the centre will have been interacting with the fungus for longer than tissues towards the edge of the pustule. As infected leaves senesce and become increasingly chlorotic, green islands appear. These are a characteristic feature of many biotrophic infections and become apparent only during the later stages of infection, when infection sites remain green, while the remainder of the leaf senesces (Scott, 1972).

Various studies have examined the effect of biotrophic fungal pathogens on photosynthesis in localised areas of an infected leaf, usually by excising small areas of the leaf. Despite the disadvantages of using excised leaf tissue (e.g. damage to tissue and consequent physiological responses), interesting and useful data have been obtained. In the studies of Scholes and Farrar (1985) and Roberts and Walters (1988) described in the following sections, photosynthesis was measured by following oxygen evolution. Because these measurements were made under conditions of saturating CO_2 concentration, any changes observed were likely to be due to changes in chloroplasts, rather than to altered diffusion of CO_2 to chloroplasts. Scholes and Farrar (1985) carried out their experiments on pustules of the rust *Uromyces muscari* at three different developmental stages: pre-sporulation pustules, mature sporulating pustules and pustules surrounded by green islands (Fig. 3.6). Rates of photosynthesis were greatly reduced in all diseased tissues, but the largest reductions were in sporulating pustules (Fig. 3.6). In order to determine how rust infection affected the photochemical reactions within chloroplasts, Scholes and Farrar (1985) used chlorophyll fluorescence kinetics and found changes in two major parameters of chlorophyll fluorescence, F_{var} (variable fluorescence) and F_{q} (fluorescence quenching). F_{var} is a measure of the oxidation–reduction status of the electron acceptors between PS II and PS I and is a direct indicator of PS II activity (Baker, 2008). F_{q} depends on a number of factors, including the rate of re-oxidation of the primary electron acceptor Q and the plastoquinone pool, the proton and other cation electrochemical gradients across the thylakoid membranes, the ATP concentration in the external environment of the thylakoid. F_{q} is therefore a direct indicator of the ability of the thylakoids to generate electrochemical gradients across the membranes and to stimulate ATP production (Scholes & Farrar, 1985; Baker, 2008). F_{var} and F_{q} were progressively reduced in rust pustules on bluebell leaves (Fig. 3.6), suggesting that non-cyclic electron transport and general chloroplast integrity were impaired during disease development (Scholes & Farrar, 1985).

Scholes and Farrar (1985) also examined changes in photosynthesis in green island tissues from infected leaves. Rates of photosynthesis in green islands were roughly half of rates measured in control tissues but double the rates obtained in pustules. Similar results were obtained using green islands from powdery-mildew-infected barley leaves, where the apparent quantum yield of photosynthesis, thought to be a sensitive indicator of damage to the electron transport system, was reduced by 47% compared to control tissues (Coghlan & Walters, 1992). Using quantitative imaging of chlorophyll fluorescence in oat leaves infected with the crown rust fungus, *Puccinia coronata*, Scholes and Rolfe (1996) found that green island tissue was still photosynthetically active, albeit at greatly reduced rates.



Tissue	Photosynthesis	F_{var}	F_q
Control	55	26.7	32.0
Pre-sporulation pustule	28.2	16.2	14.4
Mature sporulation pustule	13.2	1.0	1.6
Green island surrounding sporulating pustule	27.8	23.3	13.9
Pustule		3.4	4.3

Fig. 3.6 Photosynthesis and chlorophyll fluorescence (F_{var} and F_q) in localised regions of bluebell leaves infected with the rust, *Uromyces muscari*. Photosynthesis was measured as the rate of oxygen evolution ($\mu\text{mol g chlorophyll}^{-1} \text{ s}^{-1}$). Scholes and Farrar (1985). Reproduced with permission of Elsevier.

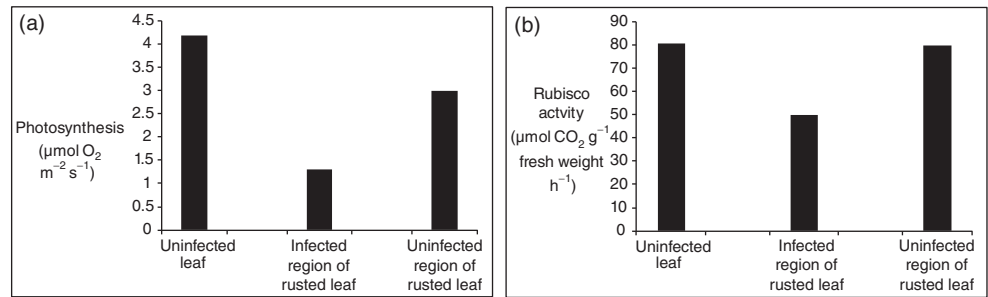


Fig. 3.7 Rates of photosynthesis (a) and Rubisco activity (b) in localised regions of leek leaves infected with rust, *Puccinia allii*. Data from Roberts and Walters (1988).

Rates of photosynthesis were also found to be greatly reduced within fungal pustules in rusted-infected leek leaves but much less so in regions between pustules (Fig. 3.7a; Roberts & Walters, 1988). These changes were accompanied by reduced activity of Rubisco in pustule regions but not in regions between pustules (Fig. 3.7b; Roberts & Walters, 1988).

In the host–pathogen systems examined in the previous paragraphs, the photosynthetic decline in infected leaves appeared to be due, in large part, to reductions in photosynthesis

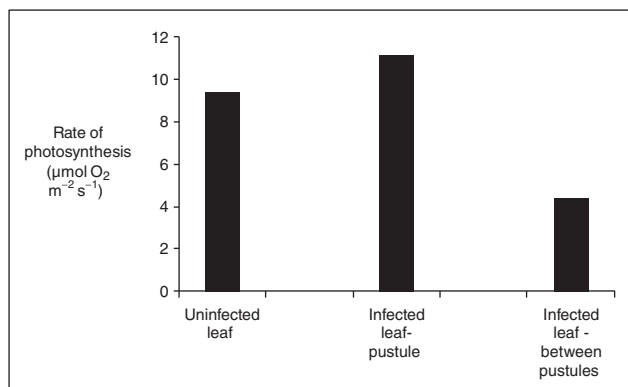


Fig. 3.8 Rates of photosynthesis in uninfected leaves of barley and in localised regions of barley leaves infected with rust, *Puccinia hordei*. Data from Scholes and Farrar (1986).

in fungal pustules, with less pronounced reductions occurring in regions between pustules. However, this situation does not hold for all host–pathogen systems. Thus, in barley leaves infected with brown rust (*P. hordei*), the decline in net photosynthesis in infected leaves was attributable largely to a reduction in photosynthetic rate in the regions between pustules (Fig. 3.8; Scholes & Farrar, 1986). In fact, the photosynthetic rate in pustules was considerably greater than rates measured either in uninfected leaves or in tissues between pustules (Fig. 3.8). The mechanism(s) underlying these changes remain unknown. However, an important lesson to take away from these studies is that it is not wise to generalise regarding different host–pathogen systems.

3.2.1.3 Changes in photosynthesis in uninfected leaves on otherwise infected plants

Most work on photosynthesis in plants infected with biotrophic fungal pathogens has been carried out on infected leaves. However, under field conditions, it is unlikely that all leaves on a plant will be infected. If this is so, then it appears reasonable to ask what happens to photosynthesis in uninfected leaves on otherwise infected plants. After all, the photosynthetic output of the whole plant will comprise rates of photosynthesis in all leaves.

Enhanced rates of net photosynthesis in uninfected leaves on otherwise infected plants have been reported from various systems, including rusted French bean (Livne & Daly, 1966), powdery-mildew-infected barley (Williams & Ayres, 1981; Walters & Ayres, 1983a), rusted leek (Roberts & Walters, 1986) and rusted broad bean (Murray & Walters, 1992). In mildewed barley, increased photosynthesis in upper, uninfected third leaves on plants with the lower two leaves infected (Fig. 3.9) was associated with an increase in the amount and activity of Rubisco (Walters & Ayres, 1983a). Interestingly, in broad bean, the increased photosynthesis in upper, uninfected leaves on plants with the two lower leaves infected with rust was accompanied by a significantly enhanced resistance of those leaves to rust infection (Fig. 3.10; Murray & Walters, 1992). Moreover, shading the upper leaves in an attempt to abolish the increase in photosynthesis in the upper, uninfected leaves reduced but did not prevent the enhanced resistance to rust infection occurring in those leaves (Table 3.1). These data suggest that, certainly in this host–pathogen system, the increased photosynthetic rates in the upper uninfected leaves on

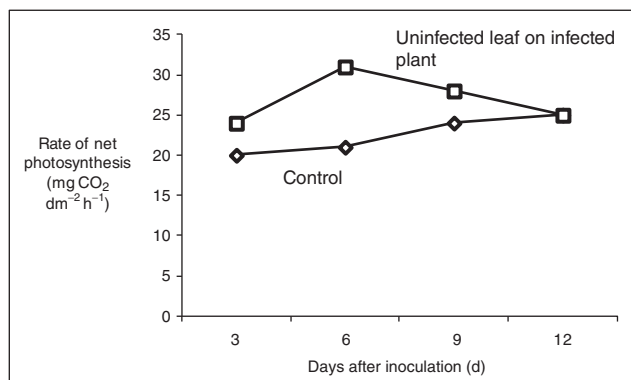


Fig. 3.9 Effects of powdery mildew infection of the lower two leaves of barley on rates of net photosynthesis in uninfected third leaves. Controls were leaves from uninfected plants. Walters and Ayres (1983). Reproduced with permission of Elsevier.

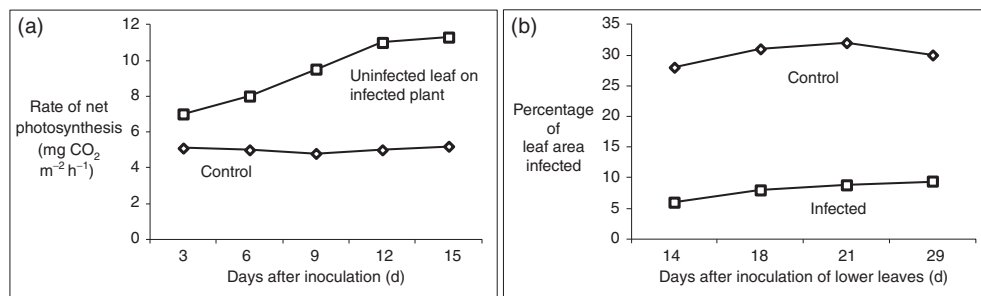


Fig. 3.10 (a) Rates of net photosynthesis in upper leaves of broad beans. In controls, the lower two leaves were not infected, while in infected plants, the lower two leaves were rust infected. In both cases, upper leaves were not infected. (b) Percentage of leaf area infected of upper leaves of broad bean after prior inoculation of the lower two leaves with rust (infected). Upper leaves were challenge inoculated with rust 1 day after inoculation of the lower leaves. In controls, the lower two leaves were not inoculated with rust. Murray and Walters (1992). Reproduced with permission of John Wiley & Sons.

otherwise infected plants is important in providing energy to finance defence reactions in those leaves (Murray & Walters, 1992).

3.2.1.4 Photosynthesis in plants infected with the clubroot pathogen

Plasmodiophora brassicae is a soil-borne, obligate parasite within the class Phytomyxea (plasmodiophorids) of the protist supergroup Rhizaria. It causes clubroot, a major disease of the family Brassicaceae. It is characterized by the development of large, club-shaped galls on the roots of susceptible plants, which give the disease its name. The formation of galls hinders the capacity of the roots to take up water and nutrients from the soil. This results in the development of above-ground symptoms in affected plants, including wilting and stunting, as well as yellowing of the leaves and premature senescence.

Table 3.1 Effect of shading the upper leaves of broad bean on the induction of systemic resistance to rust infection in those leaves. In infected plants, upper leaves were challenge inoculated 2 days after inoculation of the lower leaves. In controls, the lower leaves were not inoculated with rust.

Treatment	Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Rate of net photosynthesis ($\text{mg CO}_2 \text{m}^{-2} \text{h}^{-1}$)	Leaf area infected (%)
Control	690	6.5 ± 0.8	47.8 ± 3.1
Infected (–shade)	690	11.7 ± 1.4	3.4 ± 0.4
Infected (shade +)	500	7.6 ± 0.5	16.2 ± 2.2
Infected (shade ++)	20	0.8 ± 0.2	40.1 ± 3.9

Source: Murray and Walters (1992). Reproduced with permission of John Wiley & Sons.

Table 3.2 Photosynthetic rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) and stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$) of *Arabidopsis thaliana* and *Brassica campestris* plants infected with clubroot.

Plant species		Control	Infected
<i>A. thaliana</i>	Photosynthesis	12.12 ± 1.2	6.73 ± 0.61
	Stomatal conductance	0.37 ± 0.05	0.10 ± 0.01
<i>B. campestris</i>	Photosynthesis	7.86 ± 0.14	9.46 ± 0.97
	Stomatal conductance	0.21 ± 0.03	0.58 ± 0.13

Source: Evans and Scholes (1995). Reproduced with permission of Association of Applied Biologists and J. Scholes.

In a study of the effects of clubroot on carbon assimilation and metabolism, Evans and Scholes (1995) found different responses in *A. thaliana* and *Brassica campestris*. In *A. thaliana*, photosynthetic rate, measured at atmospheric CO_2 concentration, was reduced by 50% compared to control plants 5 weeks after inoculation (Table 3.2). This appeared to be the result of a substantial reduction in stomatal conductance. These results contrasted with those obtained with *B. campestris*, where photosynthetic rate and stomatal conductance were increased after clubroot infection (Table 3.2). Interestingly, when photosynthesis was measured at saturating CO_2 concentration, clubroot had no effect on *A. thaliana*, showing that infected plants had the same maximum capacity for photosynthesis as uninfected plants. This suggests that *P. brassicae* was not exerting a biochemical limitation on host photosynthesis in *A. thaliana* (Evans & Scholes, 1995). But what of the different photosynthetic responses shown by *A. thaliana* and *B. campestris*? According to Evans and Scholes (1995), this might have been the result of differences in architecture and position of the galls on the two plant species. In *A. thaliana*, following gall formation in hypocotyl tissue, roots of infected plants started to rot at the hypocotyl base and became detached from the plant. Such plants exhibited visible signs of wilting towards the end of the infection cycle. This was not observed in *B. campestris*, where galls formed in both root and hypocotyl tissue. It appears possible therefore that there might have been a reduction in the number of functional roots in *A. thaliana* infected with clubroot, thereby reducing water uptake and leading eventually to reduced stomatal conductance and reduced rates of photosynthesis (Evans & Scholes, 1995).

3.2.2 Photosynthesis in plants infected with hemibiotrophic and necrotrophic fungal pathogens

Many foliar pathogens reduce photosynthetic rates by destroying leaf tissue. For example, epidemics of late blight of potato, caused by the hemibiotrophic Oomycete pathogen *Phytophthora infestans*, can result in substantial defoliation, resulting in reduced rates of photosynthesis. However, loss of photosynthetic leaf area does not always lead to reductions in photosynthesis, because as we have seen in the previous sections, uninfected tissues might compensate for such losses.

Septoria tritici blotch (STB), caused by the ascomycete fungus *Mycosphaerella graminicola* (asexual stage: *Septoria tritici*), is one of the most important foliar diseases of wheat. *M. graminicola* is a hemibiotrophic pathogen, which means it is biotrophic early in the infection process, deriving its nutrition from the apoplast around living cells. During this biotrophic phase, there are no apparent symptoms. Subsequently, host tissue becomes chlorotic and then necrotic, as the pathogen kills the surrounding host cells and becomes necrotrophic (utilising dead host tissue). Infection of wheat by *M. graminicola* reduces rates of net photosynthesis, although the reduction in photosynthetic activity was greater than could be accounted for by visible STB symptoms (Shtienberg, 1992). Later work revealed that as long as no symptoms were visible, STB did not significantly affect rates of net photosynthesis. However, with the expression of symptoms, photosynthetic rates were reduced, with the reductions in photosynthesis becoming more significant as symptoms progressed from chlorotic to necrotic (Fig. 3.11; Robert *et al.*, 2006). In an attempt to quantify the effect of disease in asymptomatic areas of infected leaves, Bastiaans (1991) introduced the concept of the virtual lesion. A virtual lesion comprises a visible lesion and an adjacent area in which photosynthetic activity is negligible. The relationship between disease severity and photosynthesis is described by a single parameter, β , which is the ratio of virtual to visible lesions. The value of β indicates whether the effect of disease on photosynthesis is higher ($\beta > 1$), lower ($\beta < 1$) or equal ($\beta = 1$) to that accounted for by the area of visible symptoms on the leaf. Thus, a value of β greater than 1 is interpreted as an indication that, in addition to reducing the leaf area capable of carrying out photosynthesis, the disease also reduced photosynthesis in the green leaf tissue surrounding the visible lesion. For diseases caused by some necrotrophic pathogens, the virtual lesion could result from the production and diffusion of toxins into the area surrounding the lesion. With STB, although chlorotic symptoms were associated with a significant reduction in net photosynthesis, the effect was less than could be accounted for by the symptom area ($\beta < 1$). This suggests that chlorotic areas of these leaves are still photosynthetically active and agrees with work on powdery mildew and rust (see Section 3.2.1.2; Coghlan & Walters, 1992; Scholes & Rolfe, 1996). In contrast, photosynthesis in necrotic areas was reduced to a greater extent than could be accounted for by the symptom area ($\beta = 1.35$) (Robert *et al.*, 2006). A similar situation was found in bean (*Phaseolus vulgaris*) infected with the anthracnose pathogen, the hemibiotrophic fungus *Colletotrichum lindemuthianum* (Lopes & Berger, 2001). The values of β obtained were high ($\beta > 8$), indicating that photosynthesis in the green area beyond the necrotic symptoms was severely impaired. Rates of net photosynthesis were also strongly and negatively correlated with disease severity in two poplar (*Populus* spp.) hybrids infected with the Marssonina leaf spot fungus, *Marssonina brunnea*, with large differences in response by the two hybrids (Fig. 3.12; Erickson *et al.*, 2003). The high values of β obtained in this work indicated that impairment of photosynthetic activity extended beyond the visibly damaged leaf tissue. The

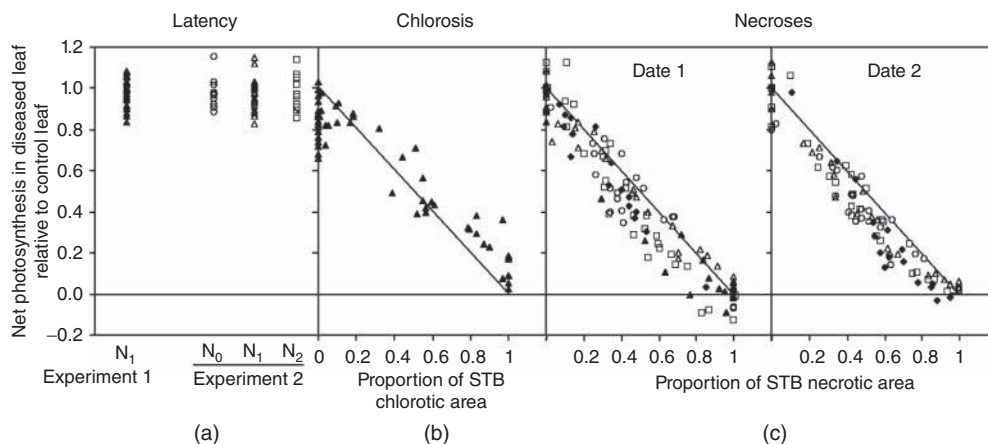


Fig. 3.11 Net photosynthetic rate in diseased leaf relative to control leaf during the development of STB. (a) 7 dai (days after inoculation), green latent tissue, measurements on flag leaves. Data are from two experiments: experiment 1 with standard fertilization level (diamonds) and experiment 2 on plants with low fertilization treatment N_0 (circles), standard fertilization treatment N_1 (triangles) and high fertilization treatment N_2 (squares). No symptoms were visible, and the x-axis represents the different treatments. (b) 13 dai, chlorotic symptoms. Data are from experiment 1: measurements on flag leaves, standard fertilization level. Line indicates $y = 1 - x$. (c) Necrotic symptoms and two assessment dates: date 1 is 20 dai and 19 dai for experiments 1 and 2, respectively; date 2 is 27 dai and 28 dai for experiments 1 (solid symbols) and 2 (open symbols), respectively. STB necrotic symptoms were assessed as the total necrotic area (including sporulating and non-sporulating necrosis). Data are from experiment 1: flag leaves (triangles) and second leaves (diamonds) and from experiment 2 for the three fertilization treatments: low fertilization treatment N_0 (circles), standard fertilization treatment N_1 (triangles) and high fertilization treatment N_2 (squares). Robert *et al.* (2006). Reproduced with permission of Oxford University Press.

reductions in photosynthesis appeared to result from disruption of the photosynthetic machinery by the pathogen (Erickson *et al.*, 2003).

It appears reasonable to expect that foliar infection will alter rates of photosynthesis. However, what should we expect following infection of roots or stems by necrotrophic pathogens? *Pythium aphanidermatum* is a major contributor to root rot of bell pepper (*Capsicum annuum*). Infected roots develop necrosis of the tips, followed by expansive browning and decay. Symptoms on aerial plant parts often include stunted shoots and fewer, smaller fruits. Inoculation of pepper plants with *P. aphanidermatum* led to reduced rates of whole plant net photosynthesis (Johnstone *et al.*, 2005). This reduction in photosynthesis was translated into a 28% reduction in cumulative carbon gain 7 days after inoculation and occurred before the appearance of visible symptoms on the shoot. The data suggested that photosynthesis was reduced as a result of reduced leaf area and was not caused by inefficient water transport from roots to the shoot (Johnstone *et al.*, 2005). Photosynthesis was also reduced in *Rhododendron macrophyllum* infected with *Phytophthora ramorum*, the cause of sudden oak death. In this case, photosynthetic capacity was reduced by 21% 3 weeks after inoculation of stems with the pathogen (Fig. 3.13; Manter *et al.*, 2007). At this stage, there were no symptoms on the leaves. One week later, at 4 weeks after inoculation, stem lesions had developed. This was accompanied by a loss in water transport capacity, leading to stomatal closure and to a further decline in photosynthetic activity (Fig. 3.13). This suggests

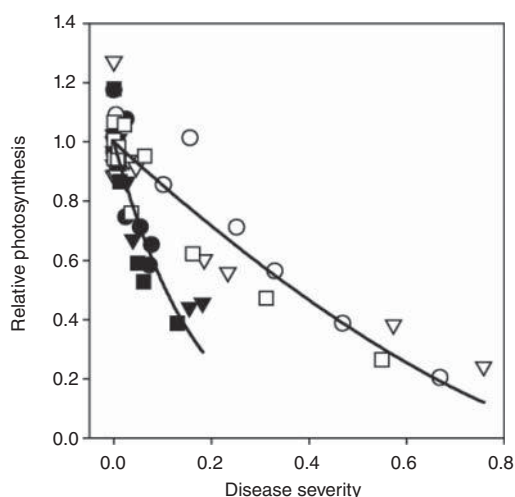


Fig. 3.12 Relative net photosynthesis (Y) of leaves in relation to disease severity (x , fraction of leaf surface with visible lesions) for two hybrid poplar clones, NM-6 (unfilled symbols) and DN-34 (filled symbols), infected with *Marssonina brunnea*. Trends in the data were described by the model $Y = (1 - x)^\beta$, which gave significantly different β -values for NM-6 ($\beta = 1.49$) and DN-34 ($\beta = 6.14$). Erickson *et al.* (2003). Reproduced with permission of John Wiley & Sons.

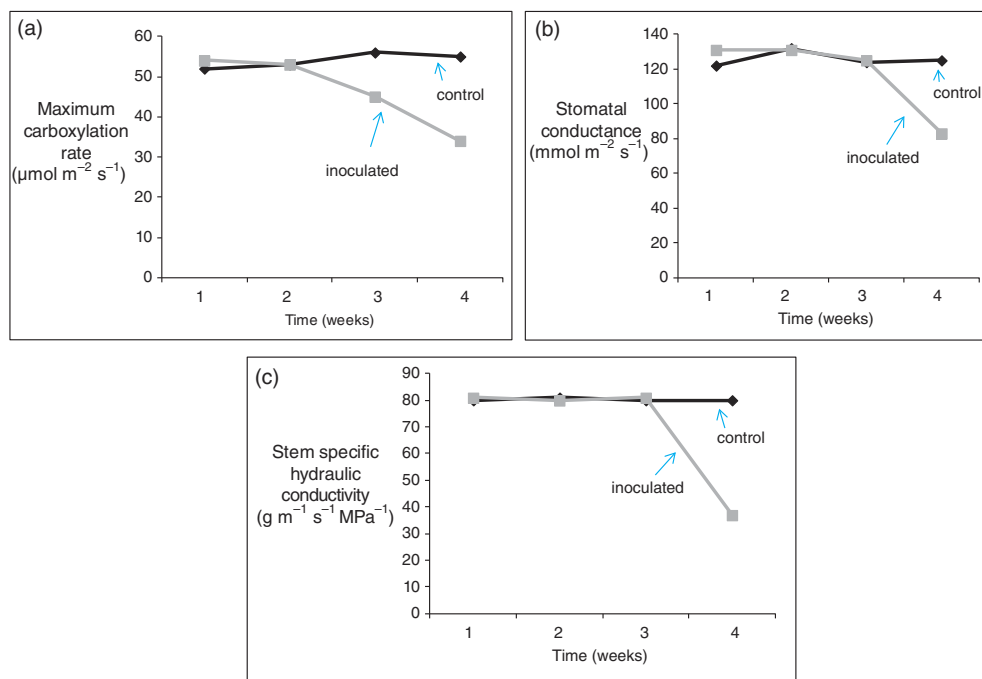


Fig. 3.13 (a) Maximum rate of carboxylation, (b) stomatal conductance and (c) stem-specific hydraulic conductivity of *Rhododendron macrophyllum* artificially inoculated with *Phytophthora ramorum*. Manter *et al.* (2007). Reproduced with permission of American Phytopathological Society.

that the reduction in photosynthesis observed in this host–pathogen system occurs in two distinct phases, an initial stage before symptom appearance and a second stage after symptom appearance. Reductions in photosynthesis in the second stage are attributable to the loss of water supply capacity associated with stem necrosis, while photosynthetic reductions in the first stage are suggestive of a toxin or a host-derived signal. Interestingly, Manter *et al.* (2007) isolated two elicitors (molecules secreted by the pathogen that manipulate host cell structure and function) from *P. ramorum*, both of which reduced photosynthetic activity in leaves of three compatible host plants. However, whether these elicitors are responsible for the reductions in photosynthesis *in planta* remains to be established.

Vascular wilt pathogens such as *Verticillium dahliae* cause water stress in host plants by reducing the hydraulic conductance of the xylem (Beckman, 1987). *V. dahliae* is the major cause of the early dying syndrome affecting potatoes in Wisconsin, USA. The syndrome is characterised by gradual leaf chlorosis, necrosis and defoliation, beginning at the base of the plant, and yield losses can be as great as 50% (Rowe *et al.*, 1987). An early symptom of this disease is a reduction in the rate of photosynthesis, which appears to be the result of water-stress-induced stomatal closure, which limits the supply of CO₂ (Fig. 3.14; Bowden *et al.*, 1990; Bowden & Rouse, 1991). *V. dahliae* also infects sunflower. However, in this plant, although photosynthesis is reduced, the effects are small and occur late (Sadras *et al.*, 2000). Indeed, the effects of *V. dahliae* on plant leaf area were first detected 31 days after inoculation, whereas effects on photosynthesis were detected 66 days after inoculation (Fig. 3.15). Moreover, the effects of infection on leaf area preceded any reduction in shoot growth, while the reduction in photosynthetic rate occurred when substantial growth reductions were already present (Fig. 3.15; Sadras *et al.*, 2000). The authors concluded that the effects of *V. dahliae* on sunflower resembled the response of the plant to water deficit, with reduced leaf expansion early in the season and accelerated leaf senescence in older plants accounting for decreased plant leaf area, and this reduced plant leaf area, rather than leaf photosynthesis, accounting for the reduced plant growth (Sadras *et al.*, 2000).

3.2.3 Photosynthesis in incompatible interactions between plants and fungal pathogens

In the previous sections, we have dealt with, for the most part, compatible interactions, that is interactions between a susceptible host and a virulent pathogen, which give rise to disease. We have only briefly mentioned incompatible interactions. In an incompatible interaction between a plant and a pathogen, resistance is generated by the rapid activation of a range of defences, including cell wall reinforcement, generation of reactive oxygen species, accumulation of pathogenesis-related proteins and phytoalexin biosynthesis (Walters, 2010). In some incompatible interactions, there is rapid localised death of host cells at the site of attempted infection, a phenomenon known as the hypersensitive response (HR). The deployment of defences requires energy and carbon skeletons. As we shall see in chapter 4, rates of dark respiration tend to increase in plants resisting pathogen challenge. However, although respiratory increases are important for defence, such changes might not be compatible with the metabolic requirements for photosynthetic carbon assimilation (Scheibe, 1991). If this is so, what happens to photosynthesis in incompatible plant–pathogen interactions?

Back in the mid-1960s, Scott and Smillie (1966) used manometric techniques to measure evolution and uptake of oxygen by leaf discs but could find no change in photosynthesis in leaves of a resistant barley cultivar inoculated with powdery mildew. Later work by Walters

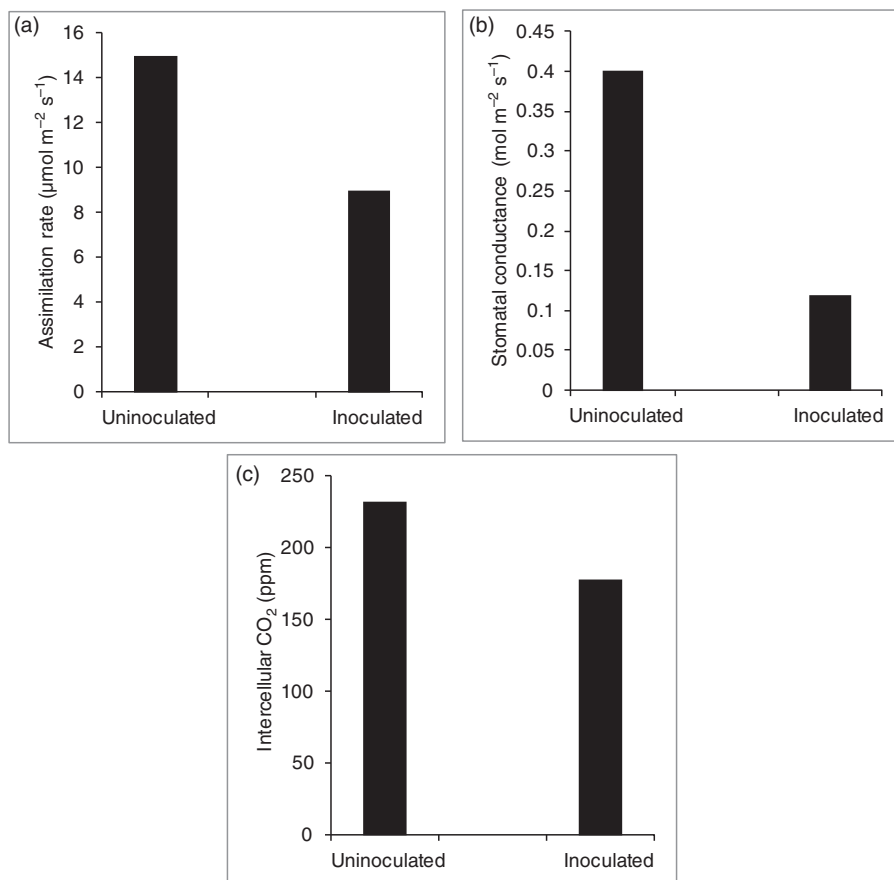


Fig. 3.14 Effects of infection by *Verticillium dahliae* on (a) assimilation rate, (b) stomatal conductance, and (c) intercellular CO_2 concentration in young potato leaves from a field experiment in 1988. Bowden and Rouse (1991). Reproduced with permission of American Phytopathological Society.

and Ayres (1983b), using whole plants, found that rates of net photosynthesis were reduced significantly in two incompatible interactions between barley and the powdery mildew fungus. Although these reductions were not long-lived, lasting just a few days, under field conditions where plants are continually challenged by pathogens, reductions in photosynthesis might be considerably more prolonged. Reductions in grain yield are known to occur in incompatible barley–powdery mildew interactions and have been associated with increased rates of dark respiration (Smedegaard-Petersen & Stolen, 1981). However, decreased photosynthetic rates could also contribute to such yield reductions.

In the work of Walters and Ayres (1983b), reductions in photosynthesis were apparent by 2 days after inoculation with powdery mildew. In tobacco reacting hypersensitively to the hemibiotrophic pathogen *Phytophthora nicotianiae*, photosynthetic activity was reduced by 6 hours after inoculation, largely as a result of stomatal closure (Fig. 3.16; Scharte *et al.*, 2005). Subsequently (>6 hours after inoculation), the photosynthetic electron chain was interrupted, and photosynthesis collapsed completely (Fig. 3.16). In this system, hypersensitive cell death

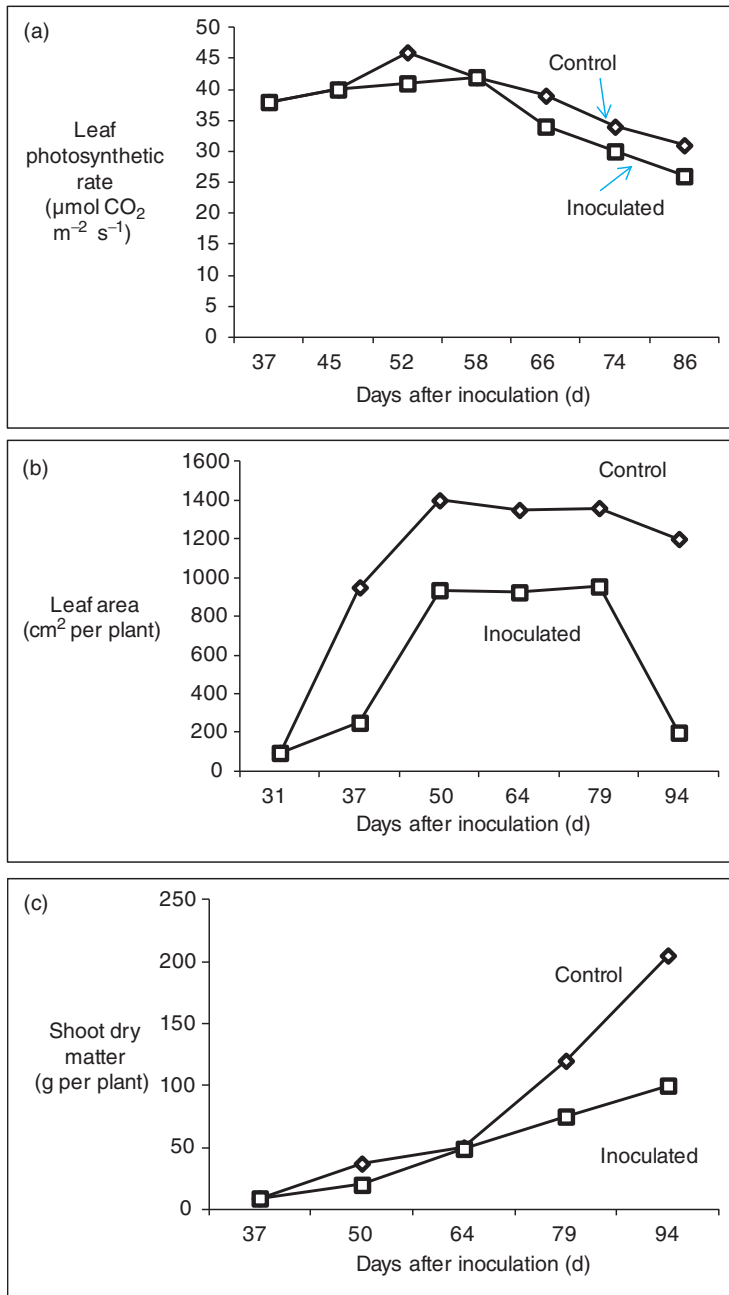


Fig. 3.15 Effects of inoculation of the sunflower hybrid Sankol with *Verticillium dahliae* on photosynthesis and growth parameters. Photosynthetic rate (a), leaf area (b), and shoot dry matter (c) of control (not inoculated) and inoculated plants. Sadras *et al.* (2000). Reproduced with permission of Oxford University Press.

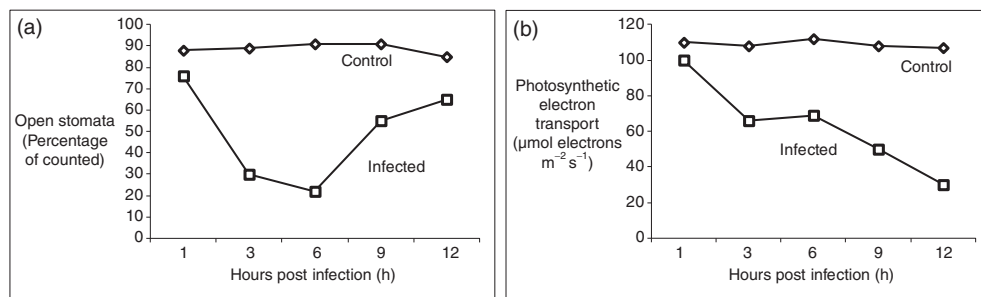


Fig. 3.16 Stomatal aperture (a) and photosynthesis (b) in an incompatible interaction between tobacco and *Phytophthora nicotianae*. The percentage of open stomata relative to total stomata was counted at the infection site. Photosynthesis was measured as changes in the capacity of photosynthetic electron transport (PET) at 2% oxygen, under which conditions, photorespiration is largely suppressed. Scharte *et al.* (2005). Reproduced with permission of John Wiley & Sons.

did not occur until photosynthesis declined completely. The authors proposed that in an incompatible host–pathogen interaction, photosynthesis and assimilatory metabolism must be switched off to initiate the increased respiration and other processes required for defence (Scharte *et al.*, 2005). In the tobacco – *P. nicotianae* interaction, reductions in photosynthesis were accompanied by changes in intercellular sugar transport and invertase activity. How alterations in carbohydrate metabolism relate to changes in photosynthetic activity and defence reactions will be dealt with fully in Chapter 5.

3.2.4 Photosynthesis in plants infected with bacterial pathogens

Infection with bacterial pathogens such as *Pseudomonas* and *Xanthomonas* spp. can lead to profound changes in photosynthetic metabolism. Over the past few years, a number of studies have examined the effects of infection by various pathovars of *Pseudomonas syringae* on photosynthesis in different hosts. *P. syringae* is a gram-negative bacterium that infects a wide variety of plants and causes necrotic symptoms in leaves, stems and fruit. It can also be found growing epiphytically and endophytically on plant foliage without causing disease symptoms (Hirano & Upper, 2000). *P. syringae* can enter the plant through natural openings such as stomata and hydathodes or through mechanical wounds. It is considered to be a hemibiotrophic pathogen because it is able to obtain nutrients from living host cells in order to multiply in the apoplast and infect neighbouring tissues. *P. syringae* pv. *tomato* DC3000 infects *A. thaliana*, and this host–pathogen system was used by Bonfig *et al.* (2006) to study the effect of virulent and avirulent strains of the bacterium on host photosynthesis. By measuring chlorophyll fluorescence parameters (maximum quantum yield of PS II and effective quantum yield of PS II), it was shown that infection with either strain of *P. syringae* reduced photosynthetic activity in *A. thaliana* (Fig. 3.17). These reductions in photosynthesis occurred before the development of visible symptoms, with changes detectable at 3 hours after inoculation with the virulent strain and 48 hours after challenge with the avirulent strain (Fig. 3.17). More detailed analysis of chlorophyll fluorescence suggested that infection by *P. syringae* exerts a direct effect on the reaction centres of PS II (Bonfig *et al.*, 2006). When photosynthetic

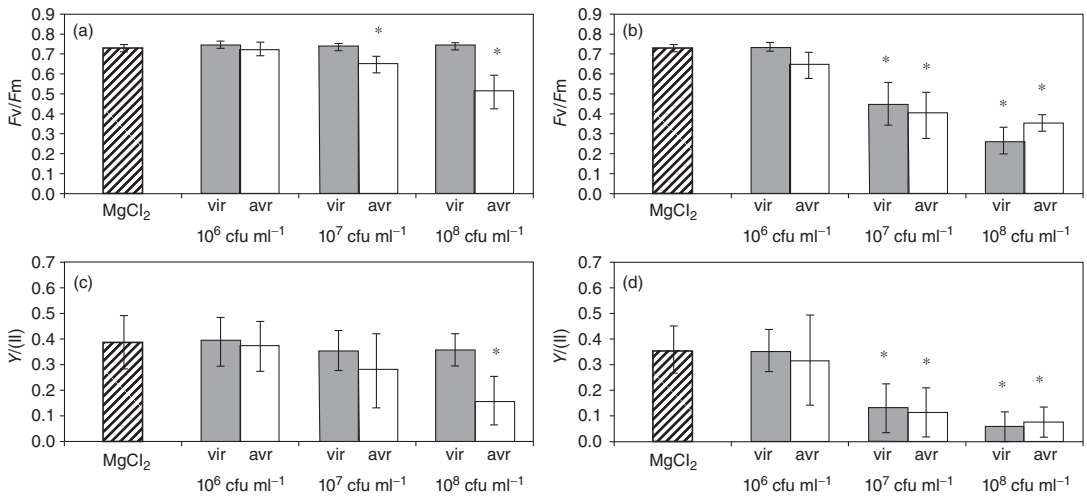


Fig. 3.17 Effects of *P. syringae* infection on the maximum quantum yield of photosystem II (F_v/F_m) and effective quantum yield of photosystem II $Y(II)$ of *Arabidopsis thaliana*. Leaves were infiltrated with the virulent (vir) or the avirulent (avr) strain of *P. syringae* or with MgCl₂ (control) and measured at (a, c) 3 h and (b, d) 24 h after infiltration. Stars indicate significant differences ($P < 0.001$) compared to the MgCl₂ treated control. Bonfig *et al.* (2006). Reproduced with permission of Springer Science + Business Media.

gene expression was examined, qualitative differences were observed between the two strains. Thus, expression of *RbcS* (encoding the small subunit of ribulose biphosphate carboxylase) and *Cab2* (encoding the chlorophyll a/b binding protein) was reduced after inoculation with the virulent strain but not the avirulent strain (Bonfig *et al.*, 2006). These findings are instructive, because although both strains reduce photosynthetic activity, only the virulent strain repressed activity of these two photosynthetic marker genes. Subsequent work by Berger *et al.* (2007) found that the plant-derived lipid signalling molecule 12-oxo-phytodienoic acid (OPDA) exerted similar effects on chlorophyll fluorescence to those resulting from *P. syringae* infection. Infiltration of *A. thaliana* leaves with OPDA led to a rapid reduction in the maximum quantum yield of PS II and, coupled with the fact that OPDA accumulates in *A. thaliana* leaves 24 hours after inoculation with *P. syringae*, suggests the involvement of OPDA in the down-regulation of photosynthesis infected leaves (Berger *et al.*, 2007).

Other pathovars of *P. syringae* also exert profound effects on photosynthesis. For example, *P. syringae* pv. *tagetis* produces a phytotoxin, tagetitoxin, during infection of many plants (Mitchell & Durbin, 1981). Infection of sunflower with this bacterial pathogen leads to a large reduction in rates of photosynthesis, accompanied by reduced stomatal conductance and large reductions in photosynthetic leaf area (Robinson *et al.*, 2004). As pointed out by Robinson *et al.* (2004), such large reductions in photosynthetic activity should not be surprising, as Rubisco activity was found to be greatly reduced in wheat leaves treated with purified tagetitoxin (Lukens & Durbin, 1985).

Xanthomonas citri pv. *citri* (Xcc) is responsible for citrus canker, one of the most devastating diseases of citrus worldwide. The bacterium produces a biologically active plant natriuretic peptide (PNP)-like protein (XacPNP), which is not present in other bacteria. PNPs are a class of extracellular, systemically mobile peptides capable of eliciting a range of plant responses that is important in homeostasis and growth. Infection of orange plants (*Citrus sinensis* cv. Valencia) with wild type Xcc and a Xcc mutant lacking XacPNP resulted in a down-regulation of photosynthesis, although the reduction in photosynthesis in plants inoculated with the XacPNP deletion mutant was markedly more dramatic (Garavaglia *et al.*, 2010; Fig. 3.18). Since the XacPNP deletion mutant died earlier in orange leaves than the wild-type bacterium, it was suggested that XacPNP is responsible for maintaining host tissue in better condition, thereby facilitating enhanced survival of the pathogen in the plant. Garavaglia *et al.* (2010) proposed that Xcc acquired and adapted a plant protein, mimicking its function to maintain host tissue in a condition better suited to its biotrophic lifestyle, for example, by directly or indirectly modulating and/or sustaining chloroplast function. Thus, when the wild type Xcc infects orange leaves, the XacPNP overcomes host necrosis earlier by counteracting the shutting down of photosynthesis, allowing the bacterium to survive for longer in the host tissue.

3.2.5 Photosynthesis in plants infected with viruses

In most plant–virus interactions, rates of photosynthesis decline as infection progresses (Balachandran *et al.*, 1997). For example, in grapevine (*Vitis vinifera* cv. Malvasia) infected with grapevine fan leaf virus (GFLV), photosynthesis was reduced by about 50% (Sampol *et al.*, 2003). Although stomatal conductance was reduced by infection, it was not responsible for the decreased photosynthesis. Furthermore, chlorophyll fluorescence analysis indicated that damage to PS II was not the main factor limiting photosynthesis in virus-infected plants. Instead, the main factor limiting photosynthesis in GFLV-infected plants was decreased carboxylation capacity, resulting from reduced activity and activation state of Rubisco (Sampol *et al.*, 2003).

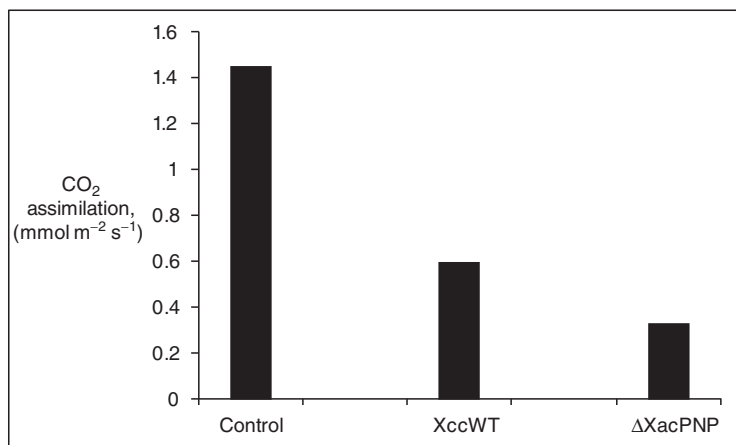


Fig. 3.18 Effect of infiltrating leaves of orange plants with the pathogenic bacterium *Xanthomonas citri* pv. *citri* (XccWT) or the bacterium lacking the PNP protein (Δ XacPNP) on photosynthesis. Measurements of CO₂ assimilation were taken 48 hours after infiltration. Garavaglia *et al.* (2010). © 2010 Garavaglia *et al.* CC BY 4.0.

Changes in photosynthesis in virus-infected plants can occur very quickly. For example, in a study of the responses of two potato cultivars, the resistant Santé and the susceptible Igor, to an aggressive isolate of *Potato Virus Y* (PVY^{NTN}), photosynthetic genes were up-regulated just 0.5 hours after inoculation (Baebler *et al.*, 2009). In the resistant Santé at this time, the up-regulation of photosynthetic genes was accompanied by an up-regulation of genes involved in chlorophyll synthesis. The authors suggested that the up-regulation might be a consequence of a general stress response triggering an increase in energy consumption. In many plant–virus interactions, reductions in photosynthesis and down-regulation of photosynthetic genes are associated with the appearance of symptoms. However, in cultivars Santé and Igor inoculated with PVY^{NTN}, there was a substantial down-regulation of photosynthetic genes (predominantly genes involved in regulating PS II) in both cultivars as soon as 12 hours after inoculation (Baebler *et al.*, 2009).

A suppression of photosynthetic activity (down-regulation of photosynthetic genes and reduction in photosynthetic rate) was also found in tomato seedlings inoculated with a mild or an aggressive isolate of the potexvirus *Pepino mosaic virus* (PepMV) (Hanssen *et al.*, 2011). It was suggested that, in addition to prioritising resources towards defence rather than primary metabolism, reduced photosynthetic activity might also protect the photosynthetic apparatus against oxidative damage or, indeed, might be a consequence of oxidative damage.

3.3 PHOTOSYNTHESIS IN PLANTS INFECTED WITH NEMATODES

Plant parasitic nematodes are agricultural pests responsible for global agricultural losses on a considerable scale. Root-knot nematodes (RKN) of the genus *Meloidogyne* include highly adapted obligate plant pathogens in temperate and tropical regions. Anatomical changes in

roots, such as giant-cell development and gall formation, are the primary symptoms of RKN infestation on susceptible plants. As we saw in Chapter 1, RKN invade the roots in the zone of elongation and then migrate intercellularly to the vascular cylinder, where they establish feeding sites and disrupt the vascular tissue (Fuller *et al.*, 2008). As a result, the water supply to the shoot is disrupted. Infestation with *M. incognita* has been shown to increase axial resistance to water flow and reduce total water uptake in tomato plants (Dorhout *et al.*, 1991). Disruptions to water transport can result in water stress, leading to above-ground symptoms such as stunting, wilting and chlorosis. Moreover, a disrupted water supply is known to affect physiological and biochemical processes such as photosynthesis and respiration (Jaleel *et al.*, 2008). There have been various reports of decreased water potential, reduced stomatal conductance and decreased photosynthetic rates in plants infested with RKN. For example, infestation of tomato by *M. ethiopica* reduced rates of photosynthesis by between 60% and 70% (Fig. 3.19; Strajnar *et al.*, 2012). This appeared to be the result of nematode-induced water stress, generated by the effects of the root galls on root hydraulic conductivity, leading to reduced leaf water potential, stomatal conductance and transpiration rates (Fig. 3.19). Interestingly, photosynthetic rates were also reduced in French beans infested with *M. incognita*, although in this case, the authors suggested that the reductions in photosynthesis were related to the lower potassium content of the leaves (Melakeberhan *et al.*, 1987). Infestation with potato cyst nematodes can also alter photosynthesis. For example, photosynthetic rates were reduced after infestation of soybean with *Heterodera glycines* (Poskuta *et al.*, 1986) and potato with the potato cyst nematode *Globodera pallida* (Schans & Arntzen, 1991).

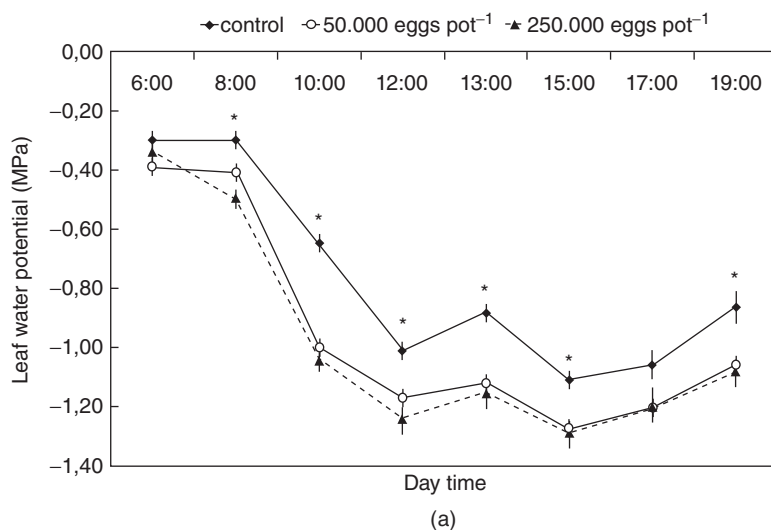


Fig. 3.19 (a) Fluctuations in leaf water potential of tomato plants inoculated with *Meloidogyne ethiopica*, 102 days after inoculation. * indicates significant differences between infested and non-infested plants. (b) Fluctuations in transpiration rate, stomatal conductance (g_s) and net photosynthesis (P_{net}) of tomato plants inoculated with *M. ethiopica*. Measurements of non-inoculated plants were significantly higher than inoculated plants in all cases. Strajnar *et al.* (2012). Reproduced with permission of Springer Science + Business Media.

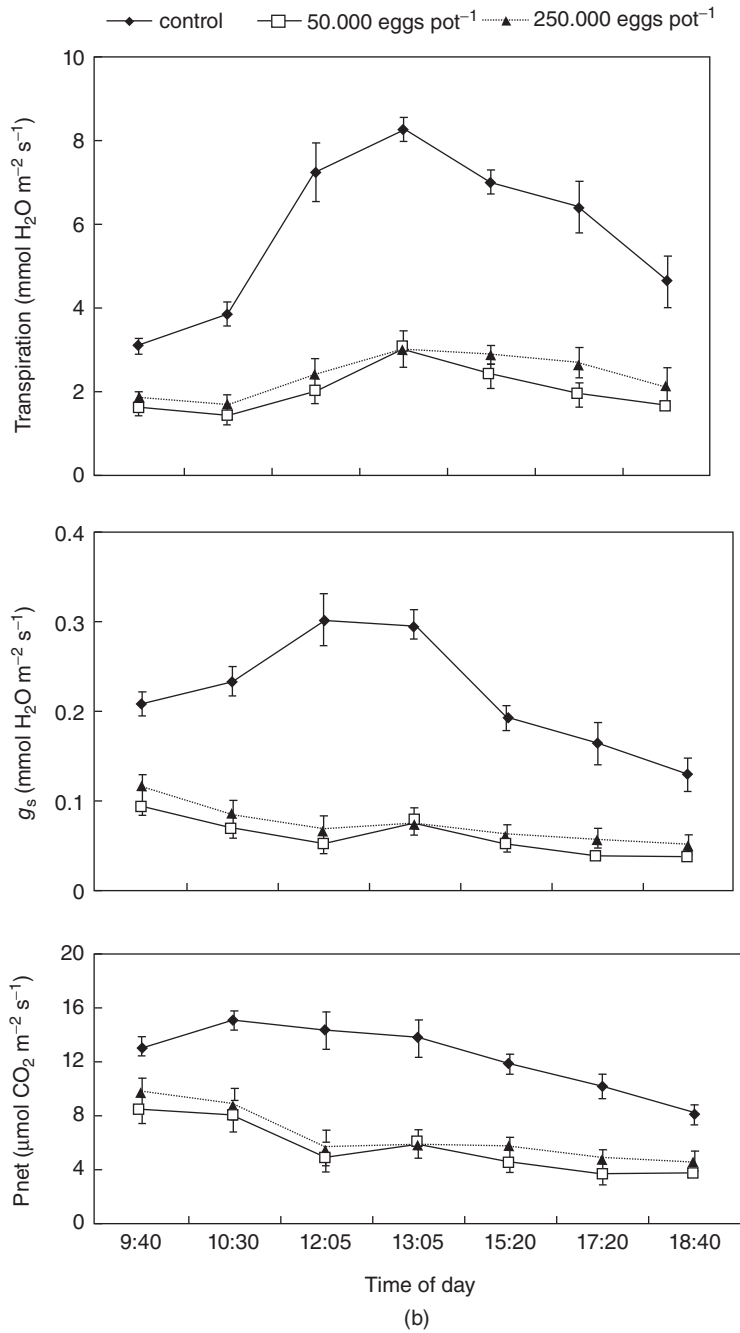


Fig. 3.19 (continued).

Root-lesion nematode disease is caused by members of the genus *Pratylenchus*. Lesion nematodes are migratory endoparasites that enter the host root to feed and reproduce and move freely through or out of the root tissue. They do not become sedentary in the roots, as do the cyst or root-knot nematodes, and feeding is restricted almost entirely to the root cortex. The root lesion nematode *P. coffeae* is a major pest of coffee in several countries, causing poor root growth, leaf loss and reductions in yield (Campos *et al.*, 1990). Infestation of coffee seedlings with these nematodes led to reductions in root and shoot fresh weight and in rates of photosynthesis (Mazzafera *et al.*, 2004; Fig. 3.20). Since these nematodes do not establish feeding sites similarly to root knot nematodes, the authors suggest that the rapid reductions in photosynthesis observed after infestation of coffee seedlings with *P. coffeae* is the result of direct damage to the root.

As mentioned in Section 3.2.2, potato early dying disease is caused primarily by the fungal pathogen *V. dahliae*. However, the root lesion nematode *P. penetrans* interacts synergistically with *V. dahliae*, resulting in enhancement of the visual symptoms of the disease and reducing

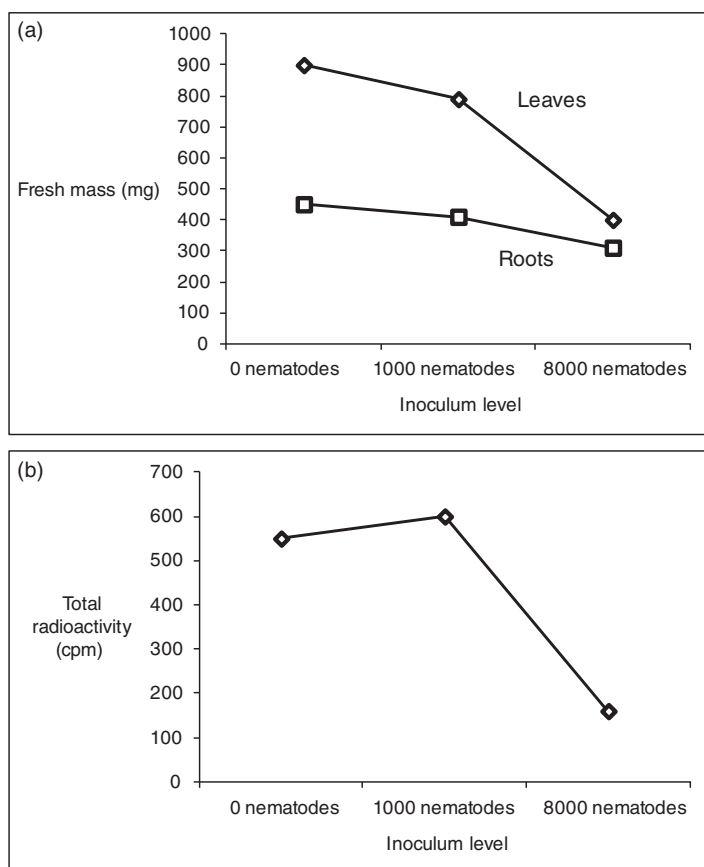


Fig. 3.20 Effect of *Pratylenchus coffeae* on growth and photosynthesis in coffee seedlings. (a) Fresh mass of leaves and roots and (b) photosynthesis (total radioactivity assimilated, expressed as counts per minute). Adapted from Mazzafera *et al.* (2004). Reproduced with permission of Springer Science + Business Media.

yield (MacGuidwin & Rouse, 1990). When potato plants grown under controlled conditions were infected with both *V. dahliae* and *P. penetrans*, photosynthesis was reduced significantly, despite the fact that there was little effect on photosynthesis when plants were inoculated with the pathogen or nematode singly (Saeed *et al.*, 1997). These reductions in photosynthesis were accompanied by reduced stomatal conductance and transpiration rates but not by a reduction in intercellular CO₂ concentration. This suggested that the reduced rates of photosynthesis in the jointly infected plants were not due simply to local plugging of the xylem vessels but more likely to a combination of stomatal and non-stomatal factors (Saeed *et al.*, 1997). Subsequent work by Saeed *et al.* (2007) found similar effects on photosynthesis over 3 years of experiments under field conditions.

3.4 PHOTOSYNTHESIS IN PLANTS INFESTED WITH INSECTS

Plant responses to insect herbivory tend to be assessed from the guild perspective, where different insect guilds are defined on the basis of their feeding mechanisms, such as chewing insects, piercing/sucking insects and so on (see Chapter 1). Welter (1989) used the guild approach to examine a substantial number of articles dealing with plant responses to insect herbivory and found that in more than half of all interactions, rates of photosynthesis were reduced. This study found that insect defoliation generally increased photosynthesis in remaining leaves, while insects feeding on cell contents tended to decrease photosynthesis (Welter, 1989). However, since that study was conducted, it has become clear that plant photosynthetic responses to insect herbivory are not as straightforward as what the analysis by Welter (1989) suggests, as we will see in the following sections.

3.4.1 Photosynthesis in plants attacked by chewing insects

Various studies have demonstrated reduced rates of photosynthesis in the remaining leaf tissue following herbivory by chewing insects. For example, in a study of photosynthesis in wild parsnip infested with caterpillars of the cabbage looper, *Trichoplusia ni*, Zangerl *et al.* (2002) found that a single caterpillar feeding for 24 hours significantly decreased the photosynthetic activity of the remaining leaf tissue. Moreover, using fluorescence imaging, they found large patches of cells where photosynthesis was depressed, well beyond the area of leaf consumed by the caterpillars. Incredibly, the indirectly affected area on the leaves was six times greater than that directly affected by tissue removal. However, because the indirectly affected area of the leaf remained photosynthetically active, albeit at a reduced rate, its contribution to the overall reductions in photosynthetic activity was only three times that of the direct effect (Zangerl *et al.*, 2002; Fig. 3.21). Later work examining the effects of *T. ni* on photosynthesis in *A. thaliana* found that the magnitude of the effects observed depended on the developmental stage of the attacking herbivore (Tang *et al.*, 2006). First instar larvae of *T. ni* feed on the underside of leaves, making small holes, avoiding leaf veins and leaving the upper epidermis intact. In contrast, fourth instar larvae make large holes, consuming minor and major veins and the leaf epidermis. Herbivory by first instar larvae reduced photosynthesis more strongly in the remaining leaf tissue than did herbivory by fourth instar larvae (Fig. 3.22). Feeding by both first and fourth instars increased transpiration in the dark substantially, and because stomata were closed, most of the water would have been lost from the cut edges of the leaf. *A. thaliana*

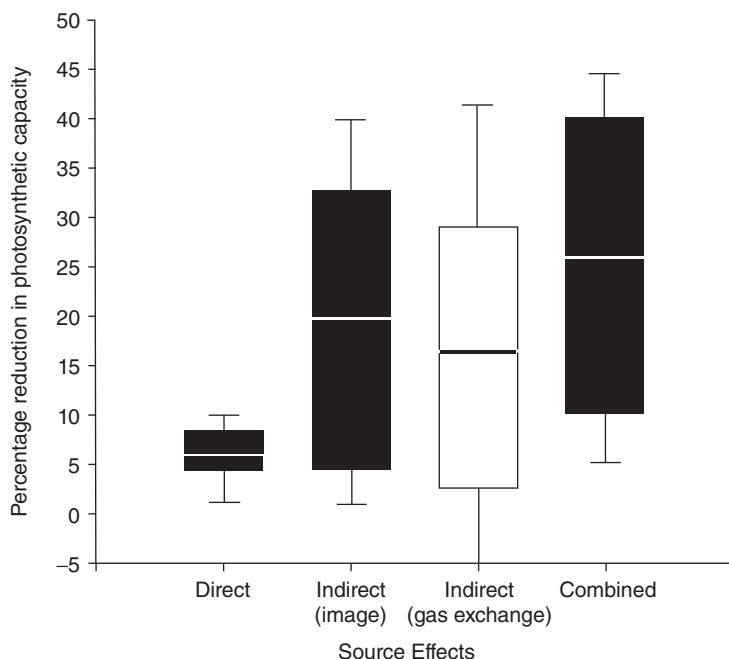


Fig. 3.21 Magnitudes of direct, indirect and total effects of caterpillar damage on suppression of photosynthesis in wild parsnip foliage. The black bars depict data obtained by fluorescence imaging, and the white bar depicts data from gas exchange. The area of leaflet measured in all cases was 6 cm^2 . The line inside each bar is the mean, the ends of the bars show the 10th and 90th percentiles, and the whiskers show the 5th and 95th percentiles. ($n = 10$.) Zangerl *et al.* (2002). Reproduced with permission of National Academy of Sciences, USA.

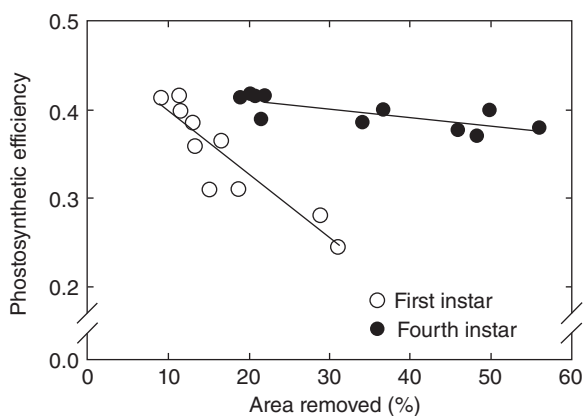


Fig. 3.22 Correlation between the proportion of *Arabidopsis* leaf tissue removed (relative to area measured) and photosystem II operating efficiency (Φ_{PSII}) of the areas of the leaf characterized as photosynthetically depressed. Leaf area with Φ_{PSII} within or below the lower 10% of the average distribution for control leaves was considered photosynthetically depressed. Each point represents a leaf on one plant, 4 days after it was exposed to herbivory. Tang *et al.* (2006). Reproduced with permission of Oxford University Press.

has reticulate vasculature, allowing water to move around sites of damage to supply nearby tissue. However, although such vasculature might be able to compensate for water loss from a large hole, it appeared to be less effective in compensating for water loss from many small holes, such as the damage resulting from first instar larvae (Tang *et al.*, 2006).

In the work of Tang *et al.* (2006), the reductions in photosynthesis appeared to be the result of localised water stress caused by damage to leaves. These workers found no change in the expression of a gene encoding the small subunit of Rubisco. However, in other plant–insect interactions, there can be substantial changes in gene expression. In the interaction between the specialist herbivore *Manduca sexta* and its natural host *Nicotiana attenuata*, there was a strong down-regulation of photosynthetic genes, accompanied by substantial up-regulation of defence-related genes (Hermsmeier *et al.*, 2001; Hui *et al.*, 2003). The latter authors suggested that the down-regulation of genes related to photosynthesis might allow attacked plants to reinvest resources into other processes, such as those involved in defence (see Box 3.1). Interestingly, a down-regulation of photosynthetic genes and an up-regulation of genes related to secondary metabolism were also found in potato leaves treated with regurgitant of the Colorado potato beetle, *Leptinotarsa decemlineata* (Lawrence *et al.*, 2008).

As indicated at the start of this section, plant photosynthetic responses to insect herbivory are not straightforward. Although there are many reports of reduced photosynthesis in remaining leaf tissue following herbivory, some of which we have just dealt with, there are also reports of increased photosynthetic activity following herbivory (e.g. Holman & Oosterhuis, 1999) or artificial defoliation (Turnbull *et al.*, 2007). Some studies have also found no effect of defoliation on photosynthesis. Thus, Peterson *et al.* (2004) studied the photosynthetic responses of several legume species to injury caused by mass consumption of leaf tissue. Photosynthesis was not significantly affected by either insect-induced or artificial defoliation. Similar results were obtained with a number of varieties of soybean and alfalfa (e.g. Peterson *et al.*, 1992; Peterson & Higley, 1996), suggesting that there is a common modality of response by legumes to mass consumption of leaf tissue by insects (Peterson *et al.*, 2004). However, since changes in photosynthesis were not detected in apple, crab apple, cucumber and tomato after mass leaf consumption by insects (Welter, 1989; Peterson *et al.*, 1996; Burkness *et al.*, 1999), the common modality of response appears not to be limited to legumes.

The studies described previously examined photosynthesis in individual plants and leaves. What happens if we move from single plants to large plant populations, such as stands of conifers or hardwood trees? Such studies are conducted to examine the impact of disturbance, such as forest fires or insect attacks on carbon cycling in forests. Insect attacks can influence net ecosystem productivity, the net uptake of CO₂ by the forest, via their impact on gross ecosystem photosynthesis (P_g) and ecosystem respiration. For example, measurements made in a hardwood forest in Wisconsin that had suffered 37% defoliation as a result of attack by forest tent caterpillars revealed a 24% reduction in P_g compared to non-outbreak years (Cook *et al.*, 2008). In British Columbia, outbreaks of the mountain pine beetle (*Dendroctonus ponderosae*) resulted in tree mortality on such a scale that effects on the carbon balance of forests in British Columbia were possible. A modelling study predicted the cumulative impact of the mountain pine beetle outbreak between 2000 and 2020 would be a net loss of 270 million tonnes of carbon (Kurz *et al.*, 2008), while estimates of P_g over the infestation area from 2002 to 2005 revealed a reduction of 10–20% compared to pre-outbreak levels (Coops & Wulder, 2010). However, a subsequent study of two lodgepole pine-dominated stands in British Columbia, an 85-year-old stand first attacked by mountain pine beetle in 2006 and a 110-year-old stand first

attacked in 2003, revealed that although net ecosystem production was negative after the initial attack, it increased substantially in the following year, as a result of increased productivity of the remaining trees and vegetation (Brown *et al.*, 2010). Indeed, subsequent measurements showed that the recovery of net ecosystem production increased rapidly in both stands, due to an increase in P_g and photosynthetic capacity (Brown *et al.*, 2012).

3.4.2 Photosynthesis in plants attacked by piercing-sucking insects

Damage to plants caused by insects with piercing-sucking mouthparts is often less evident than injury caused by insects with chewing mouthparts. Piercing-sucking insects may feed on sap of xylem, phloem or other plant cells, and their feeding site and the amount of damage they cause to plant tissues can vary greatly. For example, although some workers found no significant effect of aphid feeding on photosynthesis (e.g. on cotton; Gomez *et al.*, 2006), there are many reports of reductions in photosynthesis following feeding by piercing-sucking insects, including aphids. Thus, reductions in photosynthetic rates of up to 50% were obtained after infestation of soybean with the phloem-feeding soybean aphid, *Aphis glycines* (Macedo *et al.*, 2003). The magnitude of the reductions was surprising given the relatively low aphid densities used (e.g. ~50% reduction at aphid densities > 20/leaflet) and the fact that many of the leaves exhibited no visible symptoms of aphid damage (Macedo *et al.*, 2003).

Photosynthesis can also be affected by spider mite infestation. The feeding apparatus of spider mites consists of paired and partially fused cheliceral stylets, which they use to pierce the leaf surface and epidermis and disrupt the underlying mesophyll. Damage at the feeding site includes punctured and collapsed epidermal cells and a disrupted cuticle. The twospotted spider mite, *Tetranychus urticae*, has a stylet long enough to reach the photosynthetically active mesophyll tissue. Indeed, infestation of soybean with *T. urticae* resulted in reduced rates of photosynthesis (de Freitas Bueno *et al.*, 2009). The reduction in photosynthesis was due to reduced stomatal conductance, as no significant changes were observed in chlorophyll content or chlorophyll fluorescence parameters.

Reduced photosynthetic activity was also found in interactions between two host plants, savoy cabbage (*Brassica oleracea*) and French bean (*Phaseolus vulgaris*), and the phytophagous stink bugs, *Murgantia histrionica* and *Nezara viridula* (Velikova *et al.*, 2010). *M. histrionica* feeds using a lacerate and flush approach, which involves heavy damage to the mesophyll tissue as a result of mechanical laceration of the cells and extra-oral digestion by salivary enzymes (Miles, 1972). In contrast, *N. viridula* adults feed on leaf veins with a stylet-sheath feeding mode, destroying only a few cells and causing little mechanical damage (Miles, 1972). Rates of photosynthesis decreased rapidly in both cabbage infested with *M. histrionica* and bean infested with *N. viridula* (Fig. 3.23A and B). Although transpiration rate and stomatal conductance were reduced by 8 hours of insect feeding, the reduction in photosynthesis was greater and more rapid than the changes in these parameters (Fig. 3.23A and B). In bean infested with *N. viridula*, the substantial reduction in photosynthesis was confirmed by a large, transient inhibition in the photochemical efficiency of PS II, while damage to the maximal quantum yield of PS II was limited and transient, indicating that there was no permanent damage to the photochemistry of the infested leaves. In this interaction, there was a more complete recovery of chlorophyll fluorescence than photosynthetic rate, and so photosynthesis was not permanently impaired. On cabbage, *M. histrionica* caused visible and permanent damage to the leaf lamina, and in this case, a permanent impairment

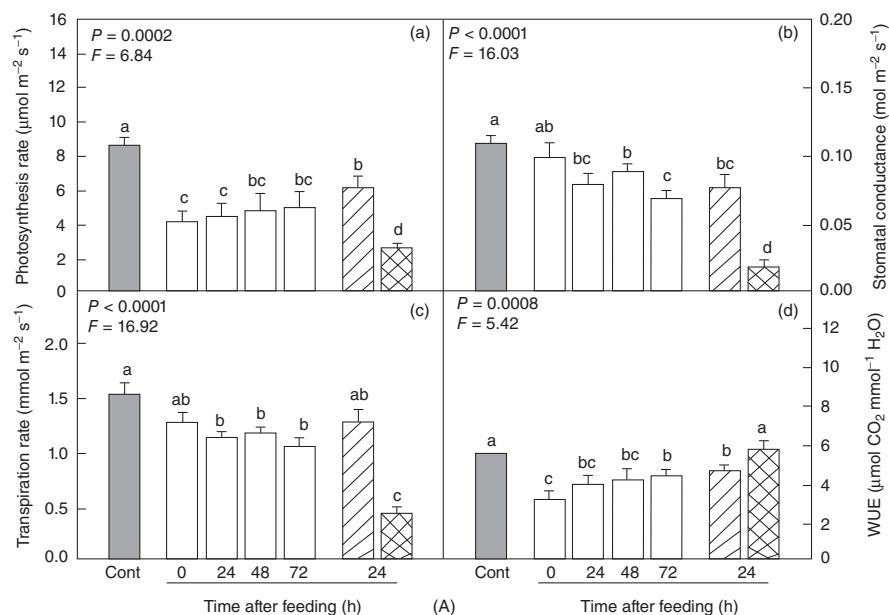


Fig. 3.23 (A) Photosynthesis rate (a), stomatal conductance (b), transpiration rate (c) and water use efficiency (WUE) (d) of savoy cabbage leaves after an 8-h long exposure to *Murgantia histrionica* adults. Bars represent control leaves (Cont, grey bars), leaves after feeding (white bars), leaves after oviposition (hatched bar), and leaves after oviposition and feeding (cross-hatched bar). Post-feeding measurements were carried out after 0, 24, 48 and 72 h, whereas in the cases of oviposition, and feeding and oviposition, measurements were carried out after 24 h only. Different letters indicate significant ($P < 0.05$) differences. (B) Photosynthesis rate (a), stomatal conductance (b), transpiration rate (c) and WUE (d) of French bean leaves after an 8-h long exposure to *Nezara viridula* adults. Bars represent control leaves (Cont, grey bars) and of leaves after feeding (white bars). Post-feeding measurements were carried out after 0, 24, 48 and 72 h. Different letters indicate significant ($P < 0.05$) differences. Velikova *et al.* (2010). Reproduced with permission of Springer Science + Business Media.

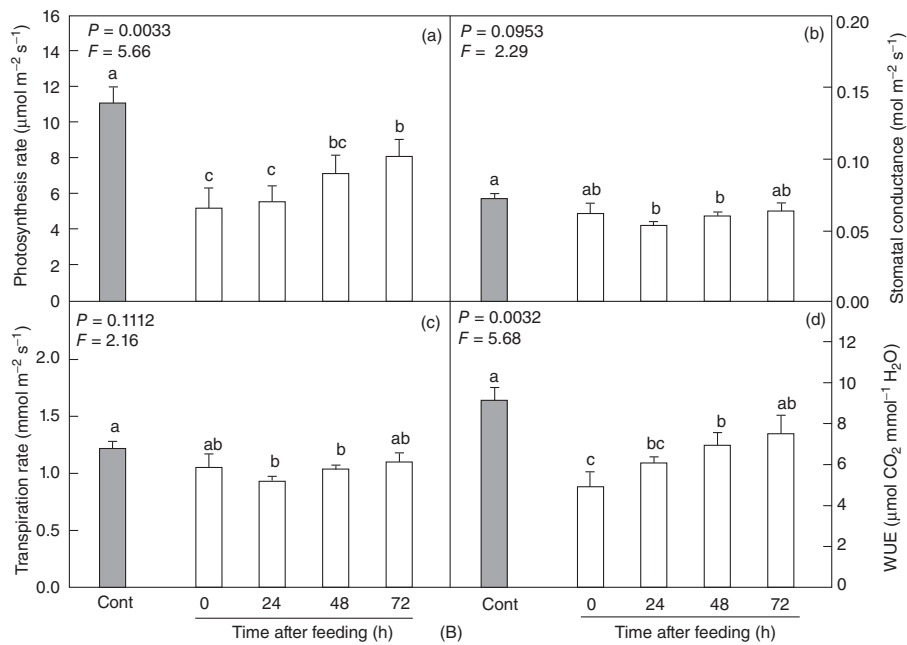


Fig. 3.23 (continued).

of photosynthetic photochemistry was found in all damaged areas of leaves. In this system, photosynthesis did recover, although the recovery was incomplete and was driven by improved photosynthetic performance in unaffected areas of leaves (Velikova *et al.*, 2010).

Compensatory increases in photosynthesis have also been obtained in plants attacked by piercing-sucking insects. Thus, photosynthetic capacity of field-grown *Pinus radiata* trees was significantly greater after artificial defoliation or infestation with the phloem-feeding aphid, *Essigella californica* (Eyles *et al.*, 2011), while compensatory increases in photosynthesis occurred in beech trees (*Northofagus solandri* var. *solandri*) infested with the scale insect, *Ultracoelostoma assimile* (Dungan *et al.*, 2007).

3.4.3 Photosynthetic changes following oviposition

Insect eggs represent a future threat to plants and the ability to detect oviposition, thereby allowing activation of appropriate defences, could provide an advantage to the host. Indeed, direct and indirect defences to oviposition have been detected, including the development of necrotic, a necrotic zone at the site of oviposition in *Brassica nigra*, leading to desiccation of the egg and mortality and the release of volatiles, attracting parasitoids of eggs (Shapiro & Devay, 1987; Meiners & Hilker, 2000; Hilker *et al.*, 2002). If oviposition can affect defences, is it possible that the process affects plant primary metabolism? This was studied by Schröder *et al.*, 2005, who examined the effects of egg deposition by the sawfly *Diprion pini* on photosynthesis in Scots pine (*Pinus sylvestris*). Sawfly adults were allowed to lay eggs on the lower section of *P. sylvestris* twigs and rates of photosynthesis measured in the upper section of the twigs and in untreated twigs. Rates of net photosynthesis in oviposition-induced pine twigs were significantly lower than in untreated twigs. Might this reduction in photosynthesis represent a trade-off between defence and photosynthetic activity, or might it simply be a consequence of oviposition and the associated wounding of tissue, resulting in localised water stress and stomatal closure (Schröder *et al.*, 2005)? Equally, given that some insects have been shown to be sensitive to CO₂ gradients (e.g. Stange *et al.*, 1995), is it possible that the egg parasitoid or the herbivorous sawfly uses the changes in photosynthesis for

Box 3.1 Photosynthesis or defence: priorities for plants under attack

Plants live in a hostile environment. They face attack from pathogens, pests and even other plants, and because they are sessile, getting up and running away is not an option. As a result, plants have evolved a formidable array of defences to deal with attackers (Walters, 2010). But defence does not come cheaply, requiring metabolic resources, including carbon, nitrogen and supplies of energy and reducing power. The problem is that plants require not only to defend themselves, but also to grow and reproduce, and the latter processes also require metabolic resources. So plants under attack face a dilemma – to grow or to reproduce. In reality, the choice might not be quite as stark as that, but there is still the need to prioritise the use of metabolic resources. Materials used to synthesise defensive compounds or build defence structures are not available for growth and reproduction unless there is turnover (Gómez & Zamora, 2002). As a result, plants mounting a robust defence might incur costs in the form of reduced growth and reproductive fitness (Zavala *et al.*, 2004).

Many of the metabolic resources for growth, reproduction and defence come from photosynthesis. As a result, there might also be a trade-off between photosynthesis and defence. For example, the production of defensive furanocoumarins in wild parsnip is associated with reduced photosynthetic rates (Zangerl *et al.*, 1997, 2002), while in soybean, infection by an avirulent isolate of the bacterial pathogen *Pseudomonas syringae* pv. *glycinea* led to a down-regulation of photosynthesis (Zou *et al.*, 2005). Tang *et al.* (2009) compared the spatial pattern of photosynthesis to that of induction of the defence-related cinnamate-4-hydroxylase (C4H) gene in *A. thaliana* attacked by *T. ni*. They found that in areas of leaves where C4H expression was up-regulated, photosynthesis was reduced. However, the reduction in photosynthesis spread further into surrounding areas of the leaf than did the expression of C4H. The authors suggested that the decrease in photosynthesis in areas of the leaf where C4H was induced might represent a trade-off between defence and photosynthesis. They also suggested that the spread of photosynthetic damage beyond areas of C4H expression might reflect damage to the photosynthetic apparatus by other defences (e.g. glucosinolates) or non-defence processes such as water stress (Tang *et al.*, 2009).

As we have seen so far in this chapter, there are a great many examples where insect attack (and indeed pathogen attack) is associated with reductions in photosynthesis. However, photosynthesis and defence are not always inversely related. For example, photosynthesis and nicotine production are positively correlated in *N. sylvestris* (Baldwin & Ohnmeiss, 1994), while in transgenic *N. tabacum* with decreased expression of transketolase, an enzyme involved in photosynthesis as well as contributing to the phenylpropanoid pathway, photosynthesis and accumulation of defensive compounds are reduced (Henkes *et al.*, 2001). Interestingly, it has been observed that plant genotypes that are able to maintain photosynthetic activity under insect challenge exhibit greater tolerance to attack. Thus, tolerant lines of barley and wheat attacked by the Russian wheat aphid were able to maintain photosynthetic rates, or recover photosynthetic capacity more rapidly, compared to susceptible lines (Haile *et al.*, 1999; Franzen *et al.*, 2007; Gutsche *et al.*, 2009).

Nevertheless, as we have already seen in this chapter, the expression of photosynthetically related genes is commonly down-regulated after insect attack (e.g. Hui *et al.*, 2003; Ralph *et al.*, 2006; Tang *et al.*, 2006), and compensatory increases in photosynthesis are rare (Kerchev *et al.*, 2012). Down-regulation of photosynthesis might free up resources, making them available for production of defences. It has been suggested that the rarity of compensatory increases in photosynthesis in plant–insect interactions could reflect the possibility that synthesis of secondary metabolites for defence is not costly to the plant in terms of carbon and energy, because under most conditions, plants operate to ensure a surplus of carbon and energy, stored as starch and other carbohydrates (Kerchev *et al.*, 2012).

There is a cost to everything in life. Of course, defence is costly. It requires metabolic resources after all. Whether the costs incurred by mounting defences become large enough to be measured is likely to depend on the growth conditions plants find themselves in. Under optimal conditions, such costs might be too small to detect, whereas under limiting conditions, defence costs might well be more apparent. Plants have a prodigious anabolic potential that allows them to throw just about everything at attackers in order to defend themselves (Schwachtje & Baldwin, 2008). Moreover, plants are very plastic, a trait that might well be part of its defensive armoury (Karban *et al.*, 1997).

orientation or to locate a host (Schröder *et al.*, 2005)? Irrespective of the possible adaptive significance of this oviposition-induced reduction in photosynthesis, it is interesting to note that in a study of oviposition by adults of *Pieris brassicae* on *A. thaliana*, gene expression profiling revealed a transcript signature that was remarkably similar to that obtained during a hypersensitive response (Little *et al.*, 2007). The changes in gene expression included the up-regulation of defence-related genes and the down-regulation of genes associated with growth and photosynthesis.

3.5 PHOTOSYNTHESIS IN PLANTS INFECTED WITH PARASITIC PLANTS

As we saw in Chapter 2, parasitic plants can lower growth and reproductive output of their hosts. They can also affect host photosynthesis in a variety of ways and at a range of scales, from the leaf through to the whole plant. The impact of parasitic plants on host photosynthesis depends on the species of parasite and host and, importantly, cannot always be correlated with the degree of dependence of the parasite on the host. Broadly, the effects of parasitic plants on host photosynthesis can be classified in terms of direct effects on removal of host resources (source–sink interactions) and indirect or non-source–sink interactions (Watling & Press, 2001). In this section, we look at the effects of a range of different parasitic plants on photosynthesis in their hosts and examine the mechanisms responsible for the changes observed.

3.5.1 Photosynthesis in plants infected with hemiparasites

Striga hermonthica is a root hemiparasite of C₃ and C₄ cereals in the semi-arid tropics and typically causes stunting of host plants, with associated reductions in growth and grain yield (Stewart & Press, 1990). The effects of *Striga* on host growth and yield may be due, in part, to its role as an additional sink for host carbon, inorganic solutes and water (Press *et al.*, 1990). However, although *S. hermonthica* is chlorophyllous, rates of photosynthesis tend to be low, while respiration rates are high. This leads to little, if any, carbon gain for the parasite, with the result that it is dependent on the host for supplies of carbon (Graves *et al.*, 1989). Various workers have reported reduced rates of photosynthesis in *Striga*-infected plants compared to uninfected controls. For example, Press *et al.* (1987) found that infection of sorghum with either *S. hermonthica* or *S. asiatica* markedly reduced photosynthetic capacity in the host. The very high rates of transpiration of the parasite predisposed the sorghum to water stress, although the resulting reduction in stomatal conductance was not sufficient to account for the *Striga*-induced reductions in photosynthesis. Later work by Frost *et al.* (1997) examined gas exchange in two varieties of sorghum infected with *S. hermonthica*, the susceptible CSH-1 and Ochuti, a variety with some tolerance to the parasite under field conditions. Growth of both varieties was reduced substantially by infection. In the susceptible CSH-1, photosynthesis was reduced in infected plants and declined over time, whereas in Ochuti, photosynthesis was not greatly affected by infection (Fig. 3.24). An examination of the kinetics of photosynthetic induction in these plants is instructive (Fig. 3.25). In both CSH-1 and Ochuti, the rate of photosynthesis, measured by CO₂ assimilation or using the chlorophyll fluorescence parameter Φ_{II} , increased rapidly in leaves of uninfected plants, reaching a steady state after 10–15 minutes. This was accompanied by a rapid increase in stomatal conductance in both

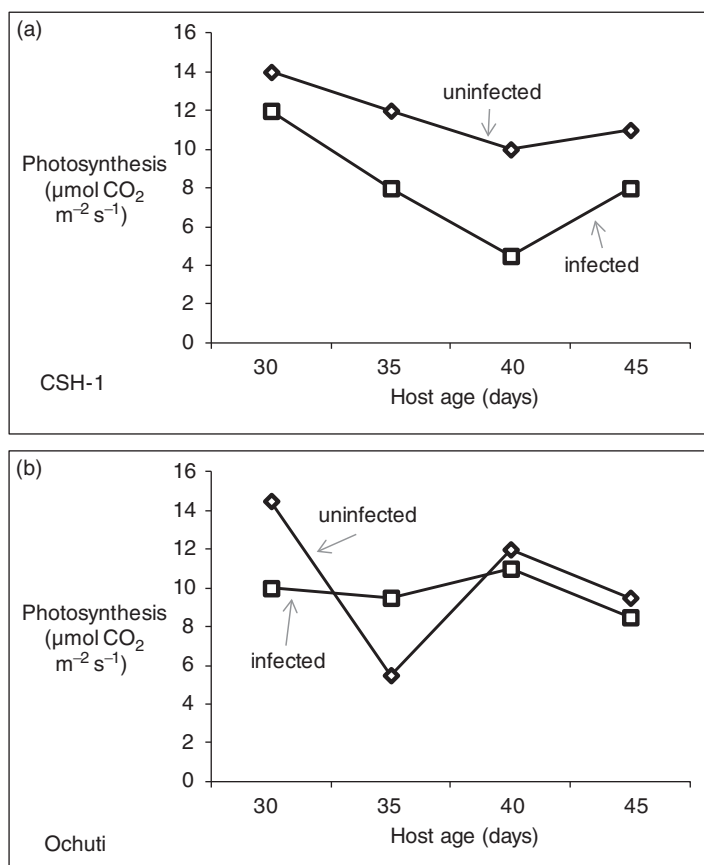


Fig. 3.24 Rate of photosynthesis of the youngest fully emerged leaf of the two sorghum cultivars and Ochuti, either uninfected or infected with *Striga hermonthica*. Frost *et al.* (1997). Reproduced with permission of John Wiley & Sons.

varieties. In leaves of CSH-1 infected with *S. hermonthica*, both photosynthesis and stomatal conductance increased slowly on irradiation and 15 minutes later had nearly reached steady state but were lower than in leaves from uninfected controls (Fig. 3.25). In *Striga*-infected Ochuti, photosynthesis and stomatal conductance also increased slowly after illumination but after some 30 minutes, reached the same values as the uninfected controls. Overall, the data provided by Frost *et al.* (1997) suggest that lower values of stomatal conductance were the primary cause of the reduced rates of photosynthesis in the *Striga*-infected sorghum. What could cause such reductions in stomatal conductance? Well, transpiration rates in infected CSH-1 were lower than in uninfected controls, and this was accompanied by an increased concentration of the plant growth regulator abscisic acid (ABA) in the xylem sap and leaf tissue of infected CSH-1 plants. Since ABA is known to be involved in controlling stomatal responses to various environmental stresses, including drought (Wilkinson & Davies, 2002), it is possible that the increased ABA found in the xylem sap and leaves of CSH-1 was responsible for the lower stomatal conductance.

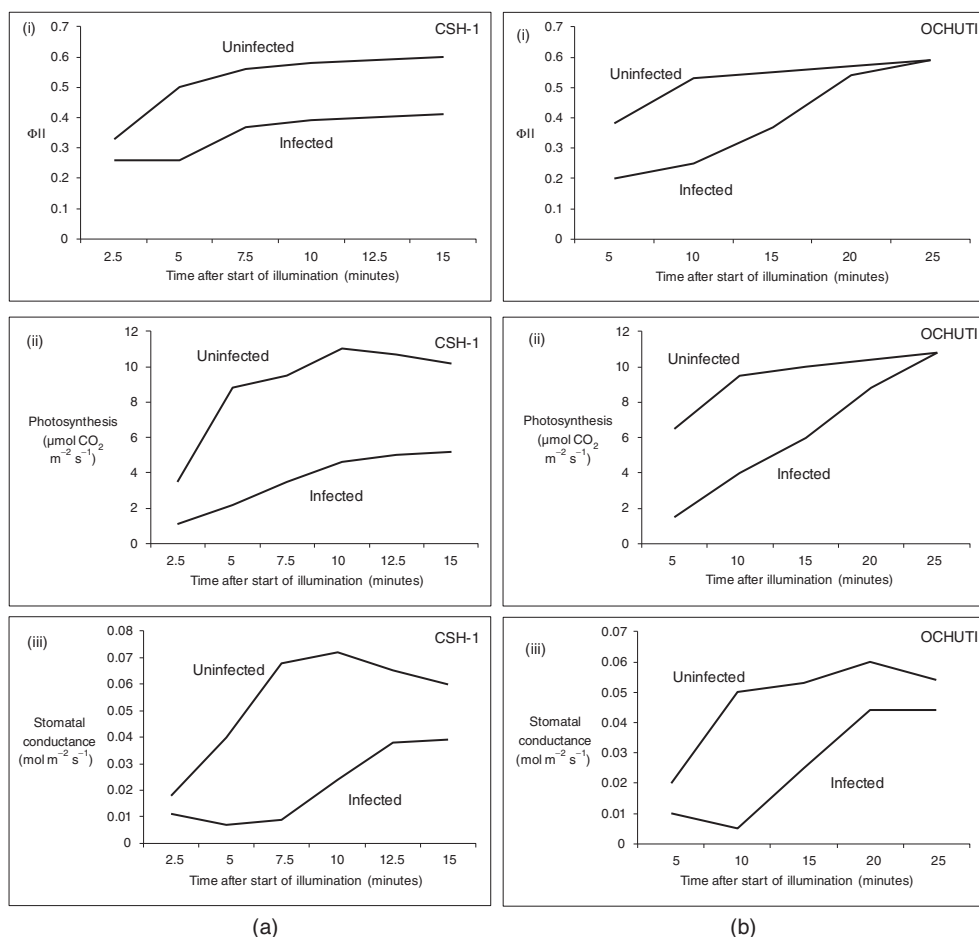


Fig. 3.25 Photochemical quenching of chlorophyll fluorescence (Φ_{II}), rate of photosynthesis and stomatal conductance of the youngest fully emerged leaf of the two sorghum cultivars CSH-1 and Ochuti, irradiated at $900 \mu\text{mol m}^{-2} \text{ s}^{-1}$ following a period of dark adaptation. Plants were 40 days old and were either uninfected or infected with *Striga hermonthica*. Note the difference in scale of the x-axis between the two cultivars. Frost *et al.* (1997). Reproduced with permission of John Wiley & Sons.

There is also evidence for increased photoinhibition in plants infected with *Striga*. This is important because if photoprotective mechanisms are inadequate, sustained exposure to high light could damage the photosynthetic machinery, leading to a chronic reduction in quantum yield and increased costs for repairing thylakoid proteins associated with electron transport (Demmig-Adams & Adams, 1992). Ramlan and Graves (1996) studied photoinhibition in sorghum infected with *S. hermonthica* and found that once the parasite had emerged above ground, photosynthesis declined in infected plants, which also became severely photoinhibited after exposure to high light. These plants took longer to recover from photoinhibition than uninfected plants and after 24 hours, still had lower quantum yields than uninfected plants, suggesting that they had sustained damage to their photosynthetic machinery (Ramlan & Graves, 1996).

Facultative root hemiparasites, such as *Rhinanthus minor*, often have a wide host range and may form haustorial connections with a number of different host plant species. Indeed, *R. minor* can grow on more than 20 host plants (Gibson & Watkinson, 1989), although grasses and legumes tend to be the best hosts for the parasite in terms of its growth and reproduction (Cameron *et al.*, 2006). In general, these hosts are significantly damaged by the parasite, while forbs (herbaceous species that are not graminoids) tend to remain undamaged. In the grass *Phleum bertolinii*, *R. minor* reduced biomass production significantly, while no significant effects on growth were found with the forb, *Plantago lanceolata* (Cameron *et al.*, 2008). These effects on host biomass production were mirrored by changes in photosynthetic activity. Thus, infection of *Phleum* by *R. minor* led to significant reductions in the quantum efficiency of PS II (Φ_{II}) and chlorophyll concentration, while no such effects were found in *Plantago* (Figs 3.26 and 3.27). Interestingly, when photosynthesis was measured in *R. minor* growing on the two hosts, although the two photosynthetic parameters (F_v/F_m and Φ_{II}) of the parasite growing on *Phleum* were similar to those of a healthy plant, both parameters were greatly reduced in *R. minor* growing on *Plantago* (Fig. 3.26 – F_v/F_m not shown in this figure) (Cameron *et al.*, 2008). From these data, it appears as though *Plantago* is able to suppress growth of *R. minor* by suppressing the electron transport rate (Φ_{II}).

Cassytha pubescens is a stem hemiparasite with an identical habit and morphology to plants in the holoparasitic genus *Cuscuta*. However, unlike *Cuscuta*, which is heterotrophic and phloem tapping, *C. pubescens* is autotrophic and xylem tapping. Infection with this parasite can kill the invasive weeds *Cytisus scoparius* and *Ulex europaeus* in the Mount Lofty Ranges of South Australia. Infection of *C. scoparius* with this parasite leads to significant reductions in rates of photosynthesis, and because Rubisco contents and chlorophyll concentrations were not altered by infection, the photosynthetic reductions were probably the result of lower stomatal conductance (Shen *et al.*, 2010; Fig. 3.28). Infected plants also exhibited lower efficiency of PS II across the diurnal cycle and were also more susceptible to photoinhibition. Indeed, the authors suggested that the lower photosynthetic rates, combined with increased susceptibility to photoinhibition, were likely to be responsible for the poor performance and even death of infected plants.

3.5.2 Photosynthesis in plants infected with holoparasites

As we saw in Chapter 1, holoparasites are achlorophyllous (or almost so) and as such are dependent on their hosts for their supply of carbon. *Orobanch* species are holoparasitic and not only have they lost any photosynthetic ability, but also their root system is vestigial, thereby making them reliant on their host for nitrogen and inorganic nutrients too (Parker & Riches, 1993). Moreover, *Orobanch* species can be important parasites on various dicotyledenous plants, and infection can lead to reductions in growth and yield (e.g. Barker *et al.*, 1996).

A study of tobacco infected with *O. cernua* showed that infected plants reduced growth substantially and that this growth reduction could be accounted for directly by diversion of dry matter towards the parasite (Hibberd *et al.*, 1998). This contrasts markedly with the situation in *Striga*-infected plants described in the previous section, where the parasitic plant usually accounts for only a small proportion of the difference in biomass between the host and the parasite. In tobacco infected with *O. cernua*, the effect of the parasite on host growth was directly attributable to the sink activity of the parasite, with reductions in host growth determined by the size of the parasite sink. In this system, infected plants maintained the same leaf area as uninfected plants, but leaf senescence was delayed. Indeed, although the rate of

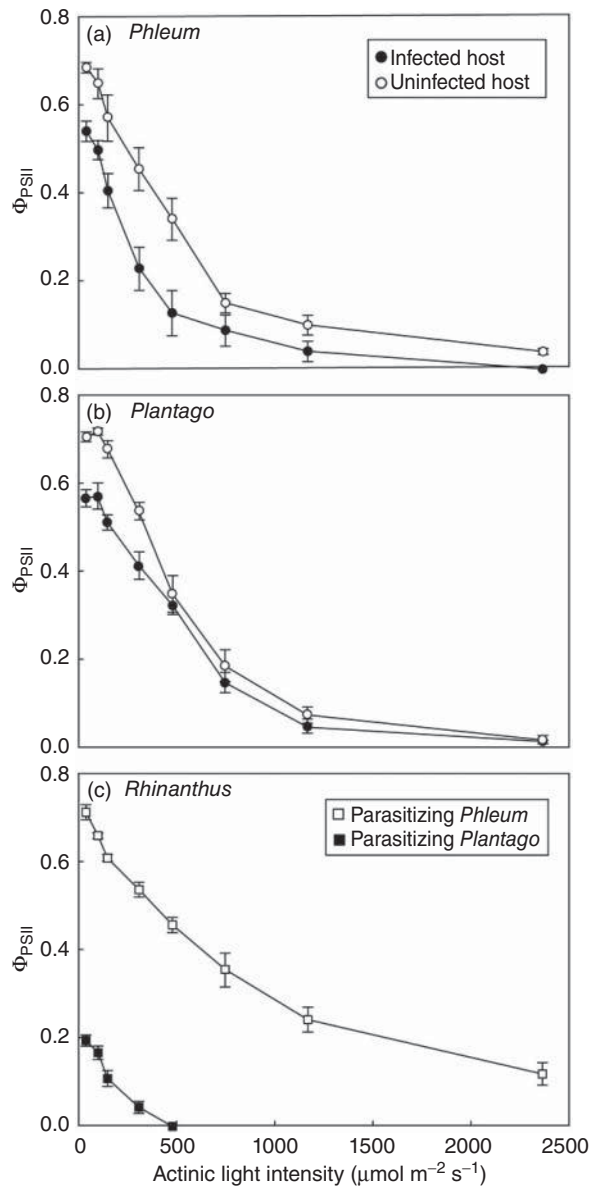


Fig. 3.26 Quantum efficiency of photosystem II (Φ_{PSII}) for host plants either infected or uninfected by the parasitic plant *Rhinanthus minor*: (a) *Phleum bertolonii* and (b) *Plantago lanceolata*; and (c) Φ_{PSII} for the parasite growing with these species. Cameron *et al.* (2008). Reproduced with permission of Oxford University Press.

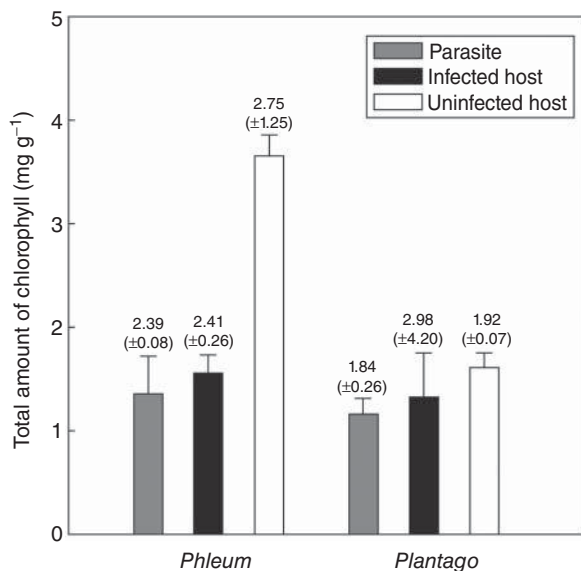


Fig. 3.27 Chlorophyll concentration in infected and uninfected host plants *Phleum bertolonii* and *Plantago lanceolata* parasitized by *Rhinanthus minor* and for the parasite growing with these species. Chlorophyll *a:b* ratios are given above the corresponding bar. Cameron *et al.* (2008). Reproduced with permission of Oxford University Press.

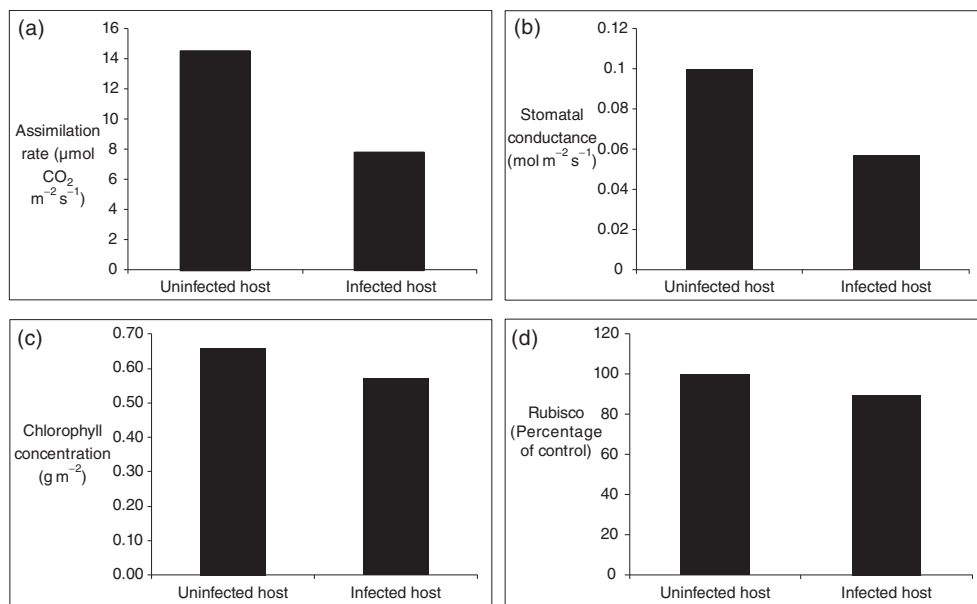


Fig. 3.28 (a) Stem photosynthesis, (b) stomatal conductance, (c) concentration of chlorophyll *a* + chlorophyll *b*, and (d) Rubisco content, for *Cytisus scoparius* plants either uninfected or infected with *Cassytha pubescens*. Shen *et al.* (2010). Reproduced with permission from CSIRO Publishing.

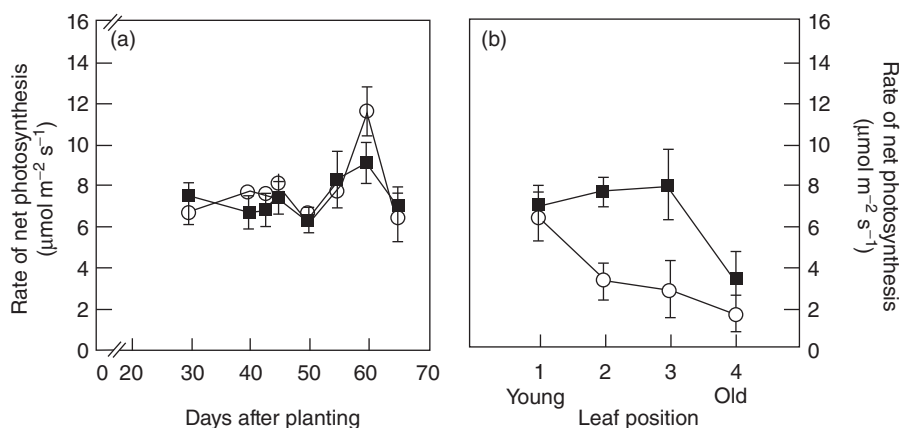


Fig. 3.29 (a) The rate of net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) of the youngest fully expanded leaf between 30 and 65 days after planting. Uninfected tobacco ($\circ$) or tobacco infected with *Orobanche cernua* ($\blacksquare$). (b) The rate of net photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$) in leaves from the top to the bottom of the canopy. Leaf positions are as follows: 1, the youngest fully expanded leaf at 65 days after planting; 2, the fourth youngest fully expanded leaf; 3, the eighth youngest fully expanded leaf; 4, the twelfth youngest fully expanded leaf. Hibberd *et al.* (1998). Reproduced with permission of John Wiley & Sons.

net photosynthesis of the youngest fully expanded leaf was not altered in infected plants, the age-dependent decline in net photosynthesis found in leaves from uninfected plants throughout the canopy was retarded in infected plants, thus maintaining their rates of photosynthesis for longer (Fig. 3.29; Hibberd *et al.*, 1998).

The parasitic angiosperm *Cuscuta* can be considered a holoparasite or an intermediate parasite, as it contains some chlorophyll, although its photosynthesis makes little contribution to its total carbon requirement (0.6%; Jeschke *et al.*, 1994). *C. reflexa* parasitizes herbaceous species such as various legumes and indeed, on one of these hosts, *Lupinus angustifolius*, it can attract as much as 82% of the current photosynthate (Jeschke *et al.*, 1994). Infection by *Cuscuta* can reduce fruit set in *L. angustifolius* substantially and can eventually kill its host. Nevertheless, infection of *L. angustifolius* with *C. reflexa* resulted in a sink-induced stimulation of host photosynthesis (Jeschke *et al.*, 1994). On the non-leguminous host *Ricinus communis*, infection by this parasite reduced host growth but did not cause irreversible damage. In this case, infection also led to a substantial, sink-dependent increase in host photosynthesis (Jeschke & Hilpert, 1997), as it did on *Coleus blumei* (Jeschke *et al.*, 1997). On the latter host, the increased photosynthesis was attributed to increased chlorophyll concentration, greater stomatal conductance, an apparent delay in leaf senescence and relief of feedback inhibition of photosynthesis (Jeschke *et al.*, 1997). Why should the effects of infection by *C. reflexa* lead to such different effects on growth of *L. angustifolius* and *Ricinus communis*? It was suggested that the severity of the effects on *L. angustifolius* might reflect the competition between the host plant, its nitrogen-fixing symbiont, *Rhizobium*, and *C. reflexa*; of course, in the non-leguminous *R. communis*, the extra competitive factor of the symbiont was not present.

Cuscuta campestris is also holoparasitic, but in contrast to *O. cernua* and *C. reflexa*, its effects on host photosynthetic activity are very different. Infection of *Mikania micrantha* with *C. campestris* can reduce host biomass substantially and can inhibit host flowering, but in this system, host photosynthesis is severely compromised. Thus, infection by *C. campestris*

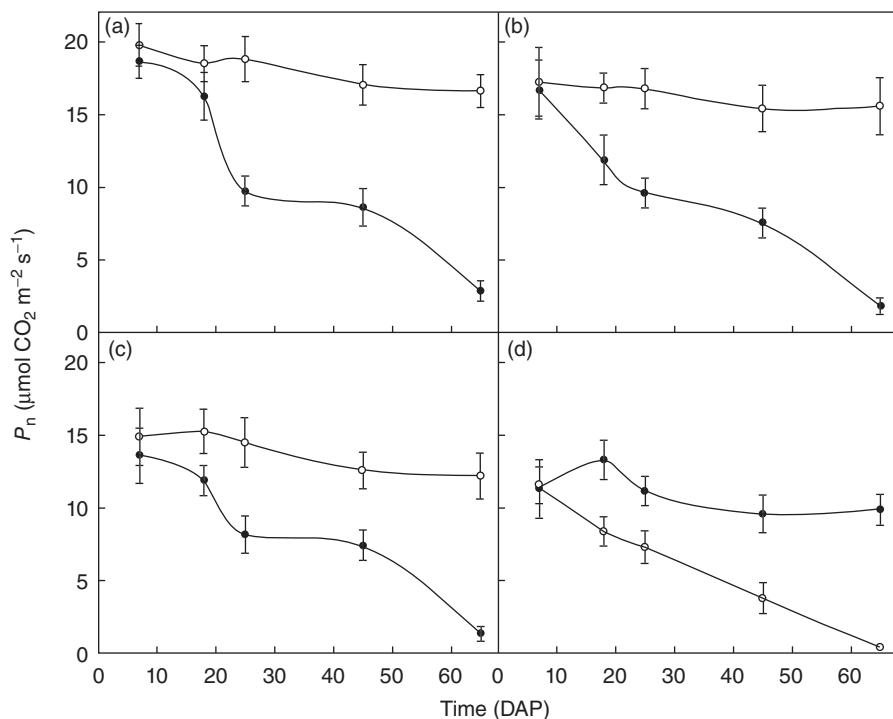


Fig. 3.30 Mean of the net photosynthetic rates (P_n) of leaves from different leaf order positions of *Mikania micrantha*, uninfected (open circles) and infected (closed circles) by *Cuscuta campestris*, between 7 and 65 days after parasitization (DAP). (a), (b), (c), and (d) are for the 1st, 4th, 8th and 12th fully expanded mature leaves, respectively. Shen *et al.* (2007). Reproduced with permission from Oxford University Press.

significantly reduced rates of net photosynthesis immediately after infection, with the inhibitory effect becoming greater with time (Shen *et al.*, 2007; Fig. 3.30). This decrease in net photosynthesis appeared to be due, in part, to lower light use efficiency and to a lower carboxylation efficiency compared to uninfected plants.

3.6 THE CARING ROBBER? HARDLY!

Parasitism is very much a one-sided affair. The parasite takes and the attacked plant has to put up with it. In most cases, this taking by the parasite has deleterious effects on the host plant. However, as Watling and Press (2001) ponder, there is surely an advantage to the parasite in keeping the host in a fit state for as long as the association lasts. Why then, do parasites tend to impair host photosynthesis and growth? Watling and Press (2001) were referring to hemiparasitic plants when they asked this question, but it also pertains to pathogens and pests. They suggested that there is no short-term disadvantage to, for example, annual hemiparasites, in reducing photosynthesis and weakening the host, because they complete their life cycle before the host dies. There can, however, be long-term implications of reducing host photosynthesis and vigour, as a negative effect on host reproductive output could put hemiparasites with high

host specificity at a disadvantage and furthermore, could exert a significant effect on community structure (Marvier & Smith, 1997; Press, 1998).

3.7 CONCLUSIONS

As we have seen in this chapter, a common reaction to parasite attack is a reduction in host photosynthesis. It has been argued that this represents a host response aimed at ensuring that resources are available for defensive actions. Whether this is true or not, it has become clear over the past few years that changes in host photosynthesis are only part of a network of interconnected metabolic events, linked closely to changes in carbohydrate metabolism and signalling. These aspects will be covered in detail in Chapter 5. In the meantime, Chapter 4 will look at changes in respiratory processes in attacked plants. Such changes are important in susceptible and resistant plants, with consequences for host growth.

RECOMMENDED READING

- Baker NR, 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annual Review of Plant Biology **59**, 89–113.
- Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S, 2006. Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. Planta **225**, 1–12.
- Cameron DD, Geniez J-M, Seel WE, Irving LJ, 2008. Suppression of host photosynthesis by the parasitic plant *Rhinanthus minor*. Annals of Botany **101**, 573–578.
- Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, De Lucia EH, 2002. Impact of herbivory on photosynthesis is greater than the sum of its holes. Proceedings of the National Academy of Sciences of the United States of America **99**, 1088–1091.

REFERENCES

- Ayres PG, 1976. Patterns of stomatal behaviour, transpiration and CO₂ exchange in pea following infection by powdery mildew (*Erysiphe pisi*). Journal of Experimental Botany **27**, 354–363.
- Baebler S, Krečič H, Rotter A, Kogovšek P, Cankar K, Kok EJ, Gruden K, Kovač M, Žel J, Pompe-Novak M, Ravnikar M, 2009. PVY^{NTN} elicits a diverse gene expression response in different potato genotypes in the first 12 h after inoculation. Molecular Plant Pathology **10**, 263–275.
- Baker NR, 2008. Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. Annual Review of Plant Biology **59**, 89–113.
- Balachandran S, Hurry VM, Kelley SE, Osmond CB, Robinson SA, Rohozinski GGR, Sims DA, 1997. Concepts of plant biotic stress. Some insights into the stress physiology of virus-infected plants, from the perspective of photosynthesis. Physiologia Plantarum **100**, 203–213.
- Baldwin IT, Ohnmeiss TE, 1994. Coordination of photosynthetic and alkaloidal responses to damage in uninducible and inducible *Nicotiana sylvestris*. Ecology **75**, 1003–1014.
- Barker ER, Press MC, Scholes JD, Quick WP, 1996. Interactions between the parasitic angiosperm *Orobancha aegyptica* and its tomato host: growth and biomass allocation. New Phytologist **133**, 637–642.
- Bastiaans L, 1991. Ratio between virtual and visual lesion size as a measure to describe reduction in leaf photosynthesis in rice due to leaf blast. Phytopathology **81**, 611–615.
- Beckman CH, 1987. *The nature of wilt diseases in plants*. St. Paul, MN, USA: APS Press.
- Berger S, Benediktyová Z, Matouš K, Bonfig K, Mueller MJ, Nedbal L, Roitsch T, 2007. Visualisation of dynamics of plant-pathogen interaction by novel combination of chlorophyll fluorescence imaging and statistical analysis: differential effects of virulent and avirulent strains of *P. syringae* and of oxylipins on *A. thaliana*. Journal of Experimental Botany **58**, 797–806.

- Bennett J, Scott KJ, 1971. Inorganic polyphosphates in the wheat stem rust fungus and in rust-infected wheat leaves. *Physiological Plant Pathology* **1**, 185–198.
- Bonfig KB, Schreiber U, Gabler A, Roitsch T, Berger S, 2006. Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. *Planta* **225**, 1–12.
- Bowden RL, Rouse DI, 1991. Effects of *Verticillium dahliae* on gas exchange of potato. *Phytopathology* **81**, 293–301.
- Bowden RL, Rouse DI, Sharkey TD, 1990. Mechanism of photosynthesis decrease by *Verticillium dahliae* in potato. *Plant Physiology* **94**, 1048–1055.
- Brown M, Black TA, Nescic Z, Foord VN, Spittlehouse DL, Fredeen AL, Grant NJ, Burton PJ, Trofymow JA, 2010. Impact of mountain pine beetle on the net ecosystem production of lodgepole pine stands in British Columbia. *Agricultural and Forest Meteorology* **150**, 254–264.
- Brown M, Black TA, Nescic Z, Fredeen AL, Foord VN, Spittlehouse DL, Bowler R, Burton PJ, Trofymow JA, Grant NJ, Lessard D, 2012. The carbon balance of two lodgepole pine stands recovering from mountain pine beetle attack in British Columbia. *Agricultural and Forest Meteorology* **153**, 82–93.
- Burkness EC, Hutchison WD, Higley LG, 1999. Photosynthetic response of ‘Carolina’ cucumber to simulated and actual striped cucumber beetle (Coleoptera: Chrysomelidae). *Entomologia Sinica* **6**, 29–38.
- Cameron DD, Coats AM, Seel WE, 2006. Host and non-host resistance underlie variable success of the hemi-parasitic plant *Rhinanthus minor*. *Annals of Botany* **98**, 1289–1299.
- Cameron DD, Geniez J-M, Seel WE, Irving LJ, 2008. Suppression of host photosynthesis by the parasitic plant *Rhinanthus minor*. *Annals of Botany* **101**, 573–578.
- Campos VP, Sivapalan P, Gnanapragasam NC, 1990. Nematode parasites of coffee, cocoa and tea. In: Luc M, Sikora RA, Bridge J, eds. *Plant parasitic nematodes in subtropical and tropical agriculture*. Wallingford, UK: CAB International, pp. 387–430.
- Coghlan SE, Walters DR, 1992. Photosynthesis in green-islands on powdery mildew-infected barley leaves. *Physiological and Molecular Plant Pathology* **40**, 31–38.
- Cook B, Bolstad P, Martin J, Heinsch F, Davis K, Wang W, Desai A, Teclaw R, 2008. Using light-use and production efficiency models to predict photosynthesis and net carbon exchange during forest canopy disturbance. *Ecosystems* **11**, 26–44.
- Coops NC, Wulder MA, 2010. Estimating the reduction in gross primary production due to mountain pine beetle infestation using satellite observations. *International Journal of Remote Sensing* **31**, 2129–2138.
- De Freitas Bueno A, de Freitas Bueno RCO, Nabity PD, Higley LG, Fernandes OA, 2009. Photosynthetic response of soybean to two spotted spider mite (Acari: Tetranychidae) injury. *Brazilian Archives of Biology and Technology* **52**, 825–834.
- Demmig-Adams B, Adams WW III, 1992. Photoprotection and other responses of plants to light stress. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**, 2029–2031.
- Doodson JK, Manners JM, Myers A, 1964. Some effects of yellow rust (*Puccinia striiformis*) on the growth and yield of spring wheat. *Annals of Botany* **28**, 459–472.
- Dorhout R, Gommers FJ, Kolloff C, 1991. Water transport through tomato roots infected with *Meloidogyne incognita*. *Phytopathology* **81**, 379–385.
- Dungan RJ, Turnbull MH, Kelly D, 2007. The carbon costs for host trees of a phloem-feeding herbivore. *Journal of Ecology* **95**, 603–613.
- Dyer TA, Scott KJ, 1972. Decrease in chloroplast polysome content of barley leaves infected with powdery mildew. *Nature* **236**, 237–238.
- Erickson JE, Stanosz GR, Kruger EL, 2003. Photosynthetic consequences of *Marssonina* leaf spot differ between two poplar hybrids. *New Phytologist* **161**, 577–583.
- Evans LJ, Scholes JD, 1995. How does clubroot alter the regulation of carbon metabolism in its host? *Aspects of Applied Biology* **42**, 125–132.
- Eyles A, Smith D, Pinkard EA, Smith I, Corkrey R, Elms S, Beadle C, Mohammed C, 2011. Photosynthetic responses of field-grown *Pinus radiata* trees to artificial and aphid-induced defoliation. *Tree Physiology* **31**, 592–603.
- Franzen LD, Gutsche AR, Heng-Moss TM, Higley LG, Sarath G, Burd JD, 2007. Physiological and biochemical responses of resistant and susceptible wheat to injury by Russian wheat aphid. *Journal of Economic Entomology* **100**, 1692–1703.
- Frost DL, Gurney AL, Press MC, Scholes JD, 1997. *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA? *Plant, Cell and Environment* **20**, 483–492.
- Fuller VL, Lilley CJ, Urwin PE, 2008. Nematode resistance. *New Phytologist* **180**, 27–44.

- Garavaglia BS, Thomas L, Gottig N, Dunger G, Garofalo CG, Daurelio LD, Ndimba B, Orellano EG, Gehring C, Ottado J, 2010. A eukaryotic acquired gene by a biotrophic phytopathogen allows prolonged survival on the host by counteracting the shut-down of plant photosynthesis. *PLoS ONE* **5** (1), 1–10.
- Gibson CC, Watkinson AR, 1989. The host range and selectivity of a parasitic plant – *Rhinanthus minor* L. *Oecologia* **78**, 401–406.
- Gómez JM, Zamora R, 2002. Thorns as induced mechanical defense in a long-lived shrub (*Hormathophylla spinosa*, Cruciferae). *Ecology* **83**, 885–890.
- Gomez SK, Oosterhuis DM, Hendrix DL, Johnson DR, Steinkraus DC, 2006. Diurnal pattern of aphid feeding and its effect on cotton leaf physiology. *Environmental and Experimental Botany* **55**, 77–86.
- Gordon TR, Duniway JM, 1982. Photosynthesis in powdery mildewed sugar beet leaves. *Phytopathology* **72**, 718–723.
- Graves JD, Press MC, Stewart GR, 1989. A carbon balance model of the sorghum-*Striga hermonthica* host-parasite association. *Plant, Cell and Environment* **12**, 101–107.
- Gutsche AR, Heng-Moss TM, Higley LG, Sarath G, Mornhinweg DW, 2009. Physiological responses of resistant and susceptible barley, *Hordeum vulgare*, to the Russian wheat aphid, *Diuraphis noxia* (Mordvilko). *Arthropod-Plant Interactions* **3**, 233–240.
- Haigh GR, Carver TLW, Gay AP, Farrar JF, 1991. Respiration and photosynthesis in oats exhibiting different levels of partial resistance to *Erysiphe graminis* D.C. ex Merat f.sp. *avenae* Marchal. *New Phytologist* **119**, 129–136.
- Haile FJ, Higley LG, Ni X, Queensberry SS, 1999. Physiological and growth tolerance in wheat to Russian wheat aphid (Homoptera: Aphididae) injury. *Environmental Entomology* **28**, 787–794.
- Hanssen IM, van Essen P, Ballester A-R, Hogewonig SW, Parra NO, Paeleman A, Lievens B, Bovy AC, Thomma BPHJ, 2011. Differential tomato transcriptomic responses induced by *Pepino Mosaic Virus* isolates with differential aggressiveness. *Plant Physiology* **156**, 301–318.
- Henkes S, Sonnewald U, Badur R, Flachmann R, Stitt M, 2001. A small decrease in plastid transketolase activity in antisense tobacco transformants has dramatic effects on photosynthesis and phenylpropanoid metabolism. *Plant Cell* **13**, 535–551.
- Hermsemeier D, Schittko U, Baldwin IT, 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology* **125**, 683–700.
- Hewitt HG, 1976. The effects of infection by *Microsphaera alphitoides* upon the growth and physiology of *Quercus robur*. *PhD Thesis, University of Lancaster*.
- Hewitt HG, Ayres PG, 1975. Changes in CO₂ and water vapour exchange rates in leaves of *Quercus robur* infected by *Microsphaera alphitoides* (powdery mildew). *Physiological Plant Pathology* **7**, 127–137.
- Hibberd JM, Quick WP, Press MC, Scholes JD, 1998. Can source-sink relations explain responses of tobacco to infection by the root holoparasitic angiosperm *Orobancha cernua*? *Plant, Cell and Environment* **21**, 333–340.
- Higgins CM, Manners JM, Scott KJ, 1985. Decrease in three messenger RNA species coding for chloroplast proteins in leaves of barley infected with *Erysiphe graminis* f.sp. *hordei*. *Plant Physiology* **78**, 891–894.
- Hilker M, Kobs C, Varma M, Schrank K, 2002. Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. *Journal of Experimental Biology* **205**, 455–461.
- Hirano SS, Upper CD, 2000. Bacteria in the leaf ecosystem with emphasis on *Pseudomonas syringae* – a pathogen, ice nucleus, and epiphyte. *Microbiology and Molecular Biology Reviews* **64**, 624–653.
- Holman EM, Oosterhuis DM, 1999. Cotton photosynthesis and carbon partitioning in response to floral bud loss due to insect damage. *Crop Science* **39**, 1347–1351.
- Hui D, Iqbal J, Lehman K, Gase K, Saluz HP, Baldwin IT, 2003. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large-scale changes in herbivore-induced mRNAs. *Plant Physiology* **131**, 1877–1893.
- Jaleel CA, Manivannan P, Lakshmanan GMA, Gomathinayagam M, Panneerselvam R, 2008. Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficit. *Colloids and Surfaces. B, Biointerfaces* **61**, 298–303.
- Jeschke WD, Hilpert A, 1997. Sink-stimulated photosynthesis, increased transpiration and increased demand-dependent stimulation of nitrate uptake: Nitrogen and carbon relations in the parasitic association *Cuscuta reflexa-Ricinus communis*. *Plant, Cell and Environment* **20**, 47–56.

- Jeschke WD, Bäuml P, R  th N, Czygan F-C, Proksch P, 1994. Modelling the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. *Journal of Experimental Botany* **45**, 801–812.
- Jeschke WD, Baig A, Hilpert A, 1997. Sink-stimulated photosynthesis, increased transpiration and increased demand-dependent stimulation of nitrate uptake: Nitrogen and carbon relations in the parasitic association *Cuscuta reflexa*-*Coleus blumei*. *Journal of Experimental Botany* **48**, 915–925.
- Johnstone M, Chatterton S, Sutton JC, Grodzinski B, 2005. Net carbon gain and growth of bell peppers, *Capsicum annuum* ‘Cubico’, following root infection by *Pythium aphanidermatum*. *Phytopathology* **95**, 354–361.
- Karban R, Agrawal AA, Mangel M, 1997. The benefits of induced defences against herbivores. *Ecology* **78**, 1351–1355.
- Kerchev PI, Fenton B, Foyer CH, Hancock RD, 2012. Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signalling pathways. *Plant, Cell and Environment* **35**, 441–453.
- Kurz WA, Dymond CC, Stinson G, Rampley GJ, Neilson ET, Carroll AL, Ebata T, Safranyik L, 2008. Mountain pine beetle and forest carbon feedback to climate change. *Nature* **452**, 987–990.
- Lawrence SD, Novak NG, Ju CJ-T, Cooke JEK, 2008. Potato, *Solanum tuberosum*, defense against Colorado potato beetle, *Leptinotarsa decemlineata* (Say): microarray gene expression profiling of potato by Colorado potato beetle regurgitant treatment of wounded leaves. *Journal of Chemical Ecology* **34**, 1013–1025.
- Little D, Gouhier-Darimont C, Bruessow F, Reymond P, 2007. Oviposition by pierid butterflies triggers defense responses in *Arabidopsis*. *Plant Physiology* **143**, 784–800.
- Livne A, Daly JM, 1966. Translocation in healthy and rust-affected beans. *Phytopathology* **56**, 170–175.
- Lopes DB, Berger RD, 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* **91**, 212–220.
- Lukens JH, Durbin RD, 1985. Tagetitoxin affects plastid development in seedling leaves of wheat. *Planta* **165**, 311–321.
- Macedo B, Bastos CS, Higley L, Ostje KR, Madhavan S, 2003. Photosynthetic responses of soybean to soybean aphid. *Journal of Economic Entomology* **96**, 188–193.
- MacGuidwin AE, Rouse DI, 1990. Role of *Pratylenchus penetrans* in the potato early dying disease of Russet Burbank potato. *Phytopathology* **80**, 1077–1082.
- Magyarosy AC, Schurmann P, Buchanan BB, 1976. Effect of powdery mildew infection on photosynthesis by leaves and chloroplasts of sugar beet. *Plant Physiology* **57**, 486–489.
- Magyarosy AC, Malkin R, 1978. Effect of powdery mildew infection of sugar beet on the content of electron carriers in chloroplasts. *Physiological Plant Pathology* **13**, 183–188.
- Manter DK, Kelsey RG, Karchesy JJ, 2007. Photosynthetic declines in *Phytophthora ramorum*-infected plants develop prior to water stress and in response to exogenous application of elicitors. *Phytopathology* **97**, 850–856.
- Marvier MA, Smith DL, 1997. Conservation implications of host use for rare parasitic plants. *Conservation Biology* **11**, 839–848.
- Mazzafera P, Kubo RK, Inomoto MM, 2004. Carbon fixation and partitioning in coffee seedlings infested with *Pratylenchus coffeae*. *European Journal of Plant Pathology* **110**, 861–865.
- Meiners T, Hilker M, 2000. Induction of plant synomones by oviposition of a phytophagous insect. *Journal of Chemical Ecology* **26**, 221–232.
- Melakeberhan H, Webster JM, Brooke RC, D’Auria JM, Cackette M, 1987. Effect of *Meloidogyne incognita* on plant nutrient concentration and its influence on the physiology of beans. *Journal of Nematology* **19**, 324–330.
- Miles PW, 1972. The saliva of Hemiptera. *Advances in Insect Physiology* **9**, 193–255.
- Mitchell RE, Durbin RD, 1981. Tagetitoxin, a toxin produced by *Pseudomonas syringae* pv. *tagetes*: purification and partial characterization. *Physiological Plant Pathology* **18**, 157–168.
- Montalbini P, Buchanan BB, 1974. Effect of a rust infection on photophosphorylation by isolated chloroplasts. *Physiological Plant Pathology* **4**, 191–196.
- Montalbini P, Buchanan BB, Hutcheson SW, 1981. Effect of rust infection on rates of photochemical oxidation and latent polyphenol oxidase activity of *Vicia faba* chloroplast membranes. *Physiological Plant Pathology* **18**, 51–57.
- Murray DC, Walters DR, 1992. Increased photosynthesis and resistance to rust infection in upper, uninfected leaves of rusted broad bean (*Vicia faba* L.). *New Phytologist* **120**, 235–242.

- Parker C, Riches CR, 1993. *Parasitic weeds of the world: biology and control*. Wallingford, UK: CAB International.
- Peterson RKD, Higley LG, 1996. Temporal changes in soybean gas exchange following simulated insect defoliation. *Agronomy Journal* **88**, 550–554.
- Peterson RKD, Danielson SD, Higley LG, 1992. Photosynthetic responses of alfalfa to actual and simulated alfalfa weevil (Coleoptera, Curculionidae) injury. *Environmental Entomology* **21**, 501–507.
- Peterson RKD, Higley LG, Spomer SM, 1996. Injury by *Hyalophora cercopia* (Lepidoptera: Saturniidae) and photosynthetic responses of apple and crabapple. *Environmental Entomology* **25**, 416–422.
- Peterson RKD, Shannon CL, Lenssen AW, 2004. Photosynthetic responses of legume species to leaf-mass consumption injury. *Environmental Entomology* **33**, 450–456.
- Poskuta JW, Dropkin VH, Nelson CJ, 1986. Photosynthesis, photorespiration and respiration of soybean after infection with root nematodes. *Photosynthetica* **20**, 405–410.
- Press MC, 1998. Dracula or Robin Hood? A functional role for root hemiparasites in nutrient poor ecosystems. *Oikos* **82**, 609–611.
- Press MC, Tuohy JM, Stewart GR, 1987. Gas exchange characteristics of the sorghum-*Striga* host parasite association. *Plant Physiology* **85**, 1143–1145.
- Press MC, Graves JD, Stewart GR, 1990. Physiology of the interaction of angiosperm parasites and their higher plant hosts. *Plant, Cell and Environment* **13**, 91–104.
- Ralph SG, Yuch H, Friedmann M, 2006. Conifer defence against insects: microarray gene expression profiling of Sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms (*Choristoneura occidentalis*) or white pine weevils (*Pissodes strobe*) reveals large scale changes of the host transcriptome. *Plant, Cell and Environment* **29**, 1545–1570.
- Ramlan MF, Graves JD, 1996. Estimation of the sensitivity to photoinhibition in *Striga hermonthica*-infected sorghum. *Journal of Experimental Botany* **47**, 71–78.
- Robert C, Bancal Marie-Odile, Lannou C, Ney B, 2006. Quantification of the effects of *Septoria tritici* blotch on wheat leaf gas exchange with respect to lesion age, leaf number, and leaf nitrogen status. *Journal of Experimental Botany* **57**, 225–234.
- Roberts AM, Walters DR, 1986. Stimulation of photosynthesis in uninfected leaves of rust-infected leeks. *Annals of Botany* **56**, 893–896.
- Roberts AM, Walters DR, 1988. Photosynthesis in discrete regions of leek leaves infected with rust. *New Phytologist* **110**, 371–376.
- Robinson JM, Lydon J, Murphy CA, Rowland R, Smith RD, 2004. Effect of *Pseudomonas syringae* pv. *tagetes* infection on sunflower leaf photosynthetic and ascorbic acid relations. *International Journal of Plant Science* **165**, 263–271.
- Rowe RC, Davis JR, Powelson ML, Rouse DI, 1987. Potato early dying: causal agents and management strategies. *Plant Disease* **71**, 482–489.
- Sadras VO, Quiroz F, Echarte L, Escande A, Pereyra VR, 2000. Effect of *Verticillium dahliae* on photosynthesis, leaf expansion and senescence of field-grown sunflower. *Annals of Botany* **86**, 1007–1015.
- Saeed IAM, MacGuidwin AE, Rouse DI, 1997. Synergism of *Pratylenchus penetrans* and *Verticillium dahliae* manifested by reduced gas exchange in potato. *Phytopathology* **87**, 435–439.
- Saeed IAM, MacGuidwin AE, Rouse DI, Malek C, 2007. A field study on the influence of *Verticillium dahliae* and *Pratylenchus penetrans* on gas exchange of potato. *Plant Disease* **91**, 1531–1535.
- Sampol B, Bota J, Riera D, Medrano H, Flexas J, 2003. Analysis of the virus-induced inhibition of photosynthesis in malmsey grapevine. *New Phytologist* **160**, 403–412.
- Schans J, Arntzen FK, 1991. Photosynthesis, transpiration and plant growth characters of different potato cultivars at various densities of *Globodera pallid*. *Netherlands Journal of Plant Pathology* **97**, 297–310.
- Scharte J, Schön H, Weis E, 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell and Environment* **28**, 1421–1435.
- Scheibe R, 1991. Redox-modulation of chloroplast enzymes. A common principle for individual control. *Plant Physiology* **96**, 1–3.
- Scholes JD, Farrar JF, 1985. Photosynthesis and chloroplast functioning within individual pustules of *Uromyces muscari* on bluebell leaves. *Physiological and Molecular Plant Pathology* **27**, 387–400.
- Scholes JD, Farrar JF, 1986. Increased rates of photosynthesis in localised regions of a barley leaf infected with brown rust. *New Phytologist* **104**, 601–612.

- Scholes JD, Farrar JF, 1987. Development of symptoms of brown rust of barley in relation to the distribution of fungal mycelium, starch accumulation and localised changes in the concentration of chlorophyll. *New Phytologist* **107**, 103–117.
- Scholes JD, Rolfe SA, 1996. Photosynthesis in localised regions of oat leaves infected with crown rust (*Puccinia coronata*): quantitative imaging of chlorophyll fluorescence. *Planta* **199**, 573–582.
- Schröder R, Forstreuter M, Hilker M, 2005. A plant notices insect egg deposition and changes its rate of photosynthesis. *Plant Physiology* **138**, 470–477.
- Schwachtje J, Baldwin IT, 2008. Why does herbivore attack reconfigure primary metabolism? *Plant Physiology* **146**, 845–851.
- Scott KJ, 1972. Obligate parasitism by phytopathogenic fungi. *Biological Reviews* **47**, 537–572.
- Scott KJ, Smillie RM, 1966. Metabolic regulation in diseased leaves. I. The respiratory rise in barley leaves infected with powdery mildew. *Plant Physiology* **41**, 289–297.
- Scott P, 2008. *Physiology and behaviour of plants*. Chichester, West Sussex: John Wiley & Sons, Ltd.
- Shapiro AM, Devay JE, 1987. Hypersensitivity reaction of *Brassica nigra* L. (Cruciferae) kills eggs of *Pieris* butterflies (Lepidoptera, Pieridae). *Oecologia* **71**, 631–632.
- Shen H, Hong L, Ye W, Cao Z, Wang Z, 2007. The influence of the holoparasitic plant *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*. *Journal of Experimental Botany* **58**, 2929–2937.
- Shen H, Prider JN, Facelli JM, Watling JR, 2010. The influence of the hemiparasitic angiosperm *Cassytha pubescens* on photosynthesis of its host *Cytisus scoparius*. *Functional Plant Biology* **37**, 14–21.
- Shtienberg D, 1992. Effects of foliar diseases on gas exchange processes: a comparative study. *Phytopathology* **82**, 760–765.
- Smedegaard-Petersen V, Stolen O, 1981. Effect of energy requiring defense reactions on yield and grain quality in a powdery mildew resistant barley cultivar. *Phytopathology* **71**, 396–399.
- Smith AM, Coupland G, Dolan L, Harberd N, Jones J, Martin C, Sablowski R, Amey A, 2010. *Plant biology*. New York: Garland Science.
- So ML, Thrower LB, 1976. The host-parasite relationship between *Vigna sesquipedalis* and *Uromyces appendiculatus*. I. Development of parasitic colonies and the pattern of photosynthesis. *Phytopathologische Zeitschrift* **85**, 320–332.
- Stange G, Munro J, Stowe S, Osmond CB, 1995. The CO₂ sense of the moth *Cactoblastus cactorum* and its probable role in the biological control of the CAM plant *Opuntia stricta*. *Oecologia* **102**, 341–352.
- Stewart GR, Press MC, 1990. The physiology and biochemistry of parasitic angiosperms. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 127–151.
- Strajnar P, Širca S, Urek G, Šircelj H, Železnik P, Vodnik D, 2012. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. *European Journal of Plant Pathology* **132**, 49–57.
- Swarbrick PJ, Schulze-Lefert P, Scholes JD, 2006. Metabolic consequences of susceptibility and resistance (race-specific and broad spectrum) in barley challenged with powdery mildew. *Plant, Cell and Environment* **29**, 1061–1076.
- Tang JY, Zielinski RE, Zangerl AR, Crofts AR, Berenbaum MR, De Lucia EH, 2006. The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *Journal of Experimental Botany* **57**, 527–536.
- Tang J, Zielinski R, Aldea M, De Lucia E, 2009. Spatial association of photosynthesis and chemical defense in *Arabidopsis thaliana* following herbivory by *Trichoplusia ni*. *Physiologia Plantarum* **137**, 115–124.
- Tang X, Rolfe SA, Scholes JD, 1996. The effect of *Albugo candida* (white blister rust) on the photosynthetic and carbohydrate metabolism of leaves of *Arabidopsis thaliana*. *Plant, Cell and Environment* **19**, 967–975.
- Turnbull TL, Adams MA, Warren CR, 2007. Increased photosynthesis following partial defoliation of field-grown *Eucalyptus globules* seedlings is not caused by increased leaf nitrogen. *Tree Physiology* **27**, 1481–1492.
- Velikova V, Salerno G, Frati F, Peri E, Conti E, Colazza S, Loreto F, 2010. Influence of feeding and oviposition by phytophagous pentatomids on photosynthesis of herbaceous plants. *Journal of Chemical Ecology* **36**, 629–641.
- Walters DR, 2010. *Plant defense: warding off attack by pathogens, herbivores and parasitic plants*. Oxford: John Wiley & Sons, Ltd.
- Walters DR, Ayres PG, 1983a. Changes in nitrogen utilisation and enzyme activities associated with CO₂ exchanges in healthy leaves of powdery mildew infected barley. *Physiological Plant Pathology* **23**, 447–459.

- Walters DR, Ayres PG, 1983b. Carbon dioxide exchange in an incompatible barley/powdery mildew combination. *Phytopathologische Zeitschrift* **107**, 176–181.
- Walters DR, Ayres PG, 1984. Ribulose biphosphate carboxylase and enzymes of CO₂ assimilation in a compatible barley/powdery mildew combination. *Phytopathologische Zeitschrift* **109**, 208–218.
- Watling JR, Press MC, 2001. Impacts of infection by parasitic angiosperms on host photosynthesis. *Plant Biology* **3**, 244–250.
- Welter SC, 1989. Arthropod impact on plant gas exchange. In: Bernays EA, ed. *Plant-insect interactions*. Boca Raton, FL, USA: CRC Press, pp. 135–150.
- Wilkinson S, Davies WJ, 2002. ABA-based chemical signalling: the coordination of responses to stress in plants. *Plant, Cell and Environment* **25**, 195–210.
- Williams GM, Ayres PG, 1981. Effects of powdery mildew and water stress on CO₂ exchanges in uninfected leaves of barley plants. *Plant Physiology* **68**, 527–530.
- Zangerl AR, Arntz AM, Berenbaum MR, 1997. Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis and growth. *Oecologia* **109**, 433–441.
- Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, De Lucia EH, 2002. Impact of folivory on photosynthesis is greater than the sum of its holes. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 1088–1091.
- Zavala JA, Patankar AG, Gase K, Baldwin IT, 2004. Constitutive and inducible trypsin proteinase inhibitor production incurs large fitness costs in *Nicotiana attenuata*. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 1607–1612.
- Zou J, Rodriguez-Zas S, Aldea M, De Lucia E, Clough SJ, 2005. Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific down regulation of photosynthesis. *Molecular Plant-Microbe Interactions* **18**, 1161–1174.

4 Respiration in Plants Interacting with Pathogens, Pests and Parasitic Plants

4.1 INTRODUCTION

Growth and maintenance of plant cells require three basic metabolic ingredients: energy, in the form of adenosine triphosphate (ATP), reducing power, usually in the form of nicotinamide adenine dinucleotide phosphate (NADP; in reduced form, NADPH), and precursor molecules. In a photosynthetic cell in the light, these requirements are met by photosynthesis, while in non-photosynthetic cells, these requirements are met mainly by carbon compounds imported from leaves (Smith *et al.*, 2010). Carbon is usually imported into non-photosynthetic tissues as sucrose, and this is metabolised initially to hexose phosphates, which in turn are metabolised further by three interrelated pathways: glycolysis, the oxidative pentose phosphate (OPP) pathway and tricarboxylic acid (TCA) cycle, also known as the Krebs cycle. These pathways provide the non-photosynthetic cell with all of their ATP, reducing power and precursor molecules. Of course, these three pathways also operate in photosynthetic tissues. The activities of these pathways are often referred to as 'dark respiration', to distinguish it from photorespiration, which is linked to photosynthesis via the unique carboxylase/oxygenase function of Rubisco (see the following section).

Before we examine the effects of attack on respiration, let us look briefly at glycolysis, the TCA cycle and the OPP pathway. Glycolysis is a cytosolic pathway that converts glucose to pyruvate and in the process, results in a small net gain of ATP (Fig. 4.1). Under aerobic conditions, phosphofructokinase (PFK) is the main regulator of glycolysis and is responsible for the formation of fructose-1,6-bisphosphate from fructose-6-phosphate. The oxidative metabolism of pyruvate by pyruvate dehydrogenase (PDH) leads to the formation of acetyl-CoA, which enters the TCA cycle (Fig. 4.1). The latter is responsible for a major portion of carbohydrate, fatty acid and amino acid oxidation, producing energy and reducing power. Under conditions that are particularly energy demanding, production of pyruvate via glycolysis can occur faster than PDH can convert it to acetyl-CoA. However, pyruvate can be used to form succinate, in a process known as the 4-aminobutyrate (GABA) shunt, thereby providing a second entry point for pyruvate into the TCA cycle and a means of utilising excess pyruvate for energy production. The TCA cycle also generates reducing equivalents (e.g. NADH) that are used

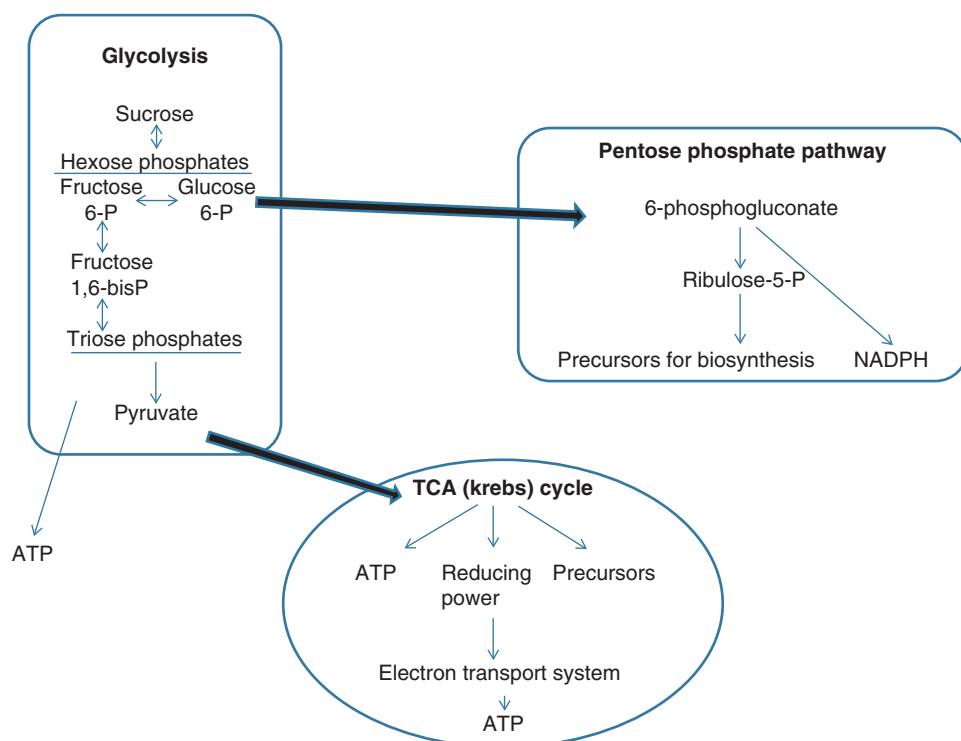


Fig. 4.1 Overview of glycolysis, the TCA cycle and the PPP. The pool of hexose phosphates, derived from sucrose, is metabolised by glycolysis and the pentose phosphate pathway to yield ATP, reducing power (NADPH and NADH), and precursors to be used in biosynthesis. Pyruvate, produced by glycolysis, moves to the mitochondrion, where it is oxidised in the TCA cycle, generating ATP, reducing power, and precursors for biosynthesis.

by the mitochondrial electron transport system to fuel the synthesis of ATP (Fig. 4.1). When flux through the TCA cycle is high, the alternative oxidase (AOX) pathway can be activated in order to channel electrons from the ubiquinone pool to form water. This allows excess energy to be lost as heat, and although it does not contribute to ATP production, it can minimise the formation of reactive oxygen species (ROS) during programmed cell death. Finally, the OPP pathway is involved in the formation of NADPH via the oxidation of glucose-6-phosphate, an intermediate shared with glycolysis (Fig. 4.1). More detailed explanations of glycolysis, the OPP pathway, the TCA cycle and the AOX pathway can be found in Öpik and Rolfe (2005) and Smith *et al.* (2010).

As indicated previously, Rubisco has both a carboxylase and an oxygenase function. Thus, in addition to carboxylating ribulose biphosphate (RuBP) with CO_2 , it can also oxygenate RuBP with oxygen to yield phosphoglycerate (PGA) and phosphoglycolate. The latter can be converted to PGA and returned to the Calvin cycle (Fig. 4.2) in a complex cycle of reactions that consumes ATP and results in the loss of one molecule of CO_2 for every molecule of PGA recovered. The loss of CO_2 that occurs during the conversion of phosphoglycolate to PGA is known as photorespiration and means that a considerable loss of photosynthate can occur.

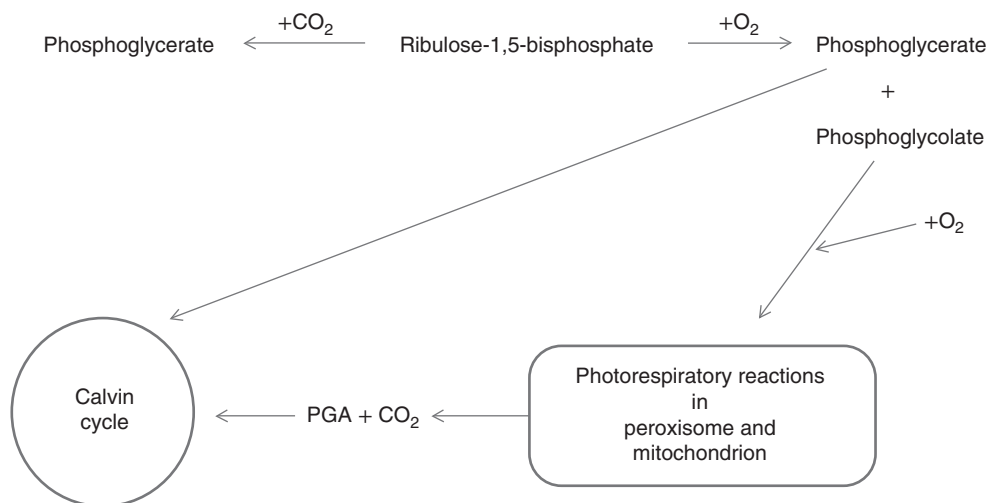


Fig. 4.2 Overview of photorespiration. Oxygenation of ribulose-1,5-bisphosphate by Rubisco gives one molecule each of 3-PGA and 2-phosphoglycolate. The latter becomes the substrate for photorespiration, with eventual loss of CO_2 , the intermediates reacting in turn with enzymes in chloroplasts, peroxisomes and mitochondria. Overall, two molecules of 2-phosphoglycolate are converted to one molecule of 3-PGA, which returns to the Calvin cycle, and one molecule of CO_2 .

Indeed, photorespiration can reduce net photosynthesis by up to 50%, although more typical values are between 15% and 25% (the reactions involved in the photorespiratory cycle are described in greater detail in Section 4.3).

Attack by pathogens, insects and parasitic plants can lead to considerable changes in both dark respiration (henceforth referred to as respiration) and photorespiration. Let us first consider effects on respiration, before turning our attention to photorespiration.

4.2 EFFECTS OF ATTACK ON RESPIRATION

4.2.1 Effects of fungal and oomycete pathogens

A common and prominent feature of fungal infection of plants is a substantial increase in respiration. Such increases can be observed in plants infected with biotrophic and necrotrophic pathogens. For example, large increases in respiration were detected in barley infected with a biotroph, *Erysiphe graminis*, and a necrotroph, *Pyrenophora teres* (Fig. 4.3; Smedegaard-Petersen, 1984). However, the kinetics of the respiratory increases was different in the two interactions. Thus, respiration rate increased quickly and peaked at about 7 days after inoculation with *P. teres* declining thereafter, as host tissues were destroyed (Fig. 4.3a). In contrast, in barley inoculated with the powdery mildew, respiration rate increased more slowly, but steadily, and continued increasing until host tissues started senescing (Fig. 4.3b). The question of whether the increased respiration in such plant–fungal interactions is of host or fungal origin has been examined in several studies using powdery mildews. Most of the powdery mildew biomass is on the leaf surface, with only the haustoria entering

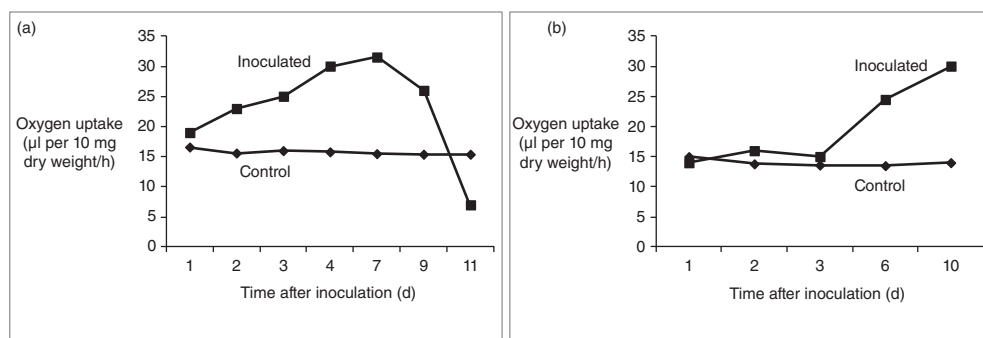


Fig. 4.3 Respiration in two susceptible barley cultivars inoculated with fungal pathogens. (a) Cultivar Wing inoculated with the necrotrophic fungus *Pyrenophora teres*, (b) Cultivar Sultan inoculated with the biotrophic fungus *Erysiphe graminis* f.sp. *hordei*. Adapted from Smedegaard-Petersen (1984). Reproduced with permission of John Wiley & Sons.

host epidermal cells. Experiments involving removal of the surface mycelium of the fungus from barley leaves have shown little change in respiration rate, suggesting that the fungal contribution to the increased respiratory rate is small (Millerd & Scott, 1956; Bushnell & Allen, 1962). This conclusion was confirmed by later work using mesophyll protoplasts isolated from barley leaves infected with powdery mildew, where the increased rate of respiration in infected tissues was attributed to the host (McAinsh *et al.*, 1989).

Respiration also increases in leaves of barley infected with brown rust (Owera *et al.*, 1981), which, analogous to powdery mildew, is biotrophic. In rust-infected barley, although respiration was increased in regions between pustules, most of the increase in respiration was localised in the pustule (Fig. 4.4; Scholes & Farrar, 1986), and indeed, similar observations

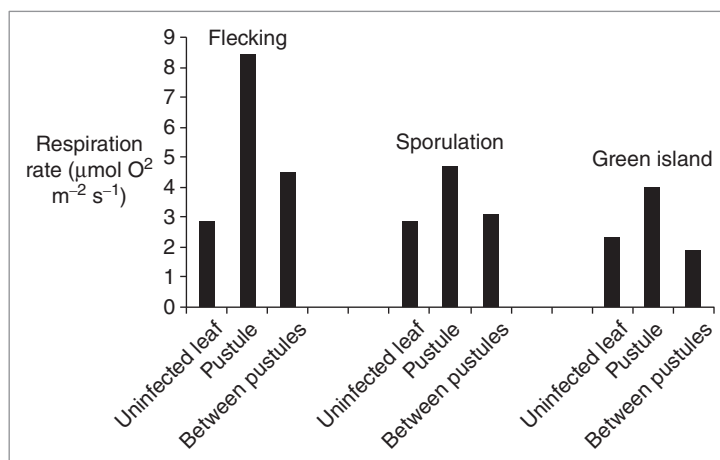


Fig. 4.4 Respiration rate in uninfected leaves and localised regions of a barley leaf infected with brown rust, at three different stages after inoculation: flecking (5 days after inoculation), sporulation (7–8 days after inoculation) and green island (9–10 days after inoculation). Data from Scholes and Farrar (1986).

were made on rusted bluebell and rusted leek (Scholes & Farrar, 1985; Roberts & Walters, 1988). Calculations of the respiratory cost of the growth of rust fungi suggested that much of the respiratory increase observed in rust-infected barley leaves should be of fungal origin (Owera *et al.*, 1981; Kneale & Farrar, 1985). It should be noted that experimental investigation of the origin of the increased rates of respiration in rust-infected tissues is not straightforward, because the pathogen grows intercellularly within leaves and produces haustoria within mesophyll cells (see Chapter 1). More recently, transcriptomic analyses have revealed up-regulation of genes involved in plant respiration in wheat–rust and poplar–rust interactions, suggesting that host respiration does increase in compatible interactions between plants and rust fungi (Bolton *et al.*, 2008; Major *et al.*, 2010).

The mechanisms underlying the increased respiration rate in powdery-mildew-infected barley were studied by Farrar and Rayns (1987). Using the susceptible cultivar Maris Otter, they observed an 80% increase in respiration, which was attributable to the host, as it was not diminished by removal of the surface mycelium. The increased respiration appeared to comprise two components: (i) increased electron flow through the cytochrome pathway in the mitochondria and (ii) an increase in electron flow through the AOX pathway. As we have seen previously, the AOX pathway is associated with a high metabolic rate and, moreover, plays a central role during plant responses to stress (Van Aken *et al.*, 2009). It is likely that it allows increased flux through glycolysis and the TCA cycle when oxidative respiration is saturated. It is therefore crucial for the production of carbon skeletons during periods of high demand (Clifton *et al.*, 2006), such as interactions between plants and pathogens.

Respiration also increases incompatible interactions between plants and pathogens, although the pattern of respiratory change differs from that observed in compatible interactions. For example, in a barley cultivar resistant to powdery mildew, respiration increases substantially just 1 day after inoculation and declines thereafter (Smedegaard-Petersen & Stolen, 1981). In contrast, and as we have seen previously, in a susceptible cultivar, respiration rate begins to increase later, some 3 days or more after inoculation (Fig. 4.3). In incompatible interactions, changes can occur very quickly indeed. Thus, in an incompatible interaction between tobacco and *Phytophthora nicotianae*, increased rates of respiration can be detected just 2 hours after infection (Fig. 4.5; Scharte *et al.*, 2005). This was accompanied by an equally rapid increase in the activity of glucose-6-phosphate dehydrogenase, a key enzyme of the OPP pathway. There appeared to be an increase in metabolites of the OPP pathway in the stroma of chloroplasts of tobacco, which could feed into defence-related biosynthesis. This is important, as the shikimic acid pathway, which is well known to be activated during defence for the synthesis of phenolics and related compounds (Herrmann & Weaver, 1999), is driven by phosphoenol pyruvate from glycolysis and erythrose-4-phosphate from the OPP pathway in the chloroplast stroma.

Interestingly, in a wheat cultivar susceptible to leaf rust caused by *Puccinia triticina*, transcriptomic analysis revealed no differentially expressed genes at 3 days after inoculation, but over 160 differentially expressed genes at 7 days after inoculation, including genes involved in respiratory metabolism (Bolton *et al.*, 2008). In contrast, in a wheat variety with partial resistance to leaf rust, mediated by the resistance gene *Lr34*, the situation was very different. In this case, there was substantial up-regulation of genes at 3 days after inoculation, including both defence genes and genes involved in respiration. Thus, genes up-regulated included those involved in the TCA cycle, glycolysis, the PDH bypass, CoA biosynthesis and the GABA shunt (Bolton *et al.*, 2008). Such a large up-regulation of genes involved in respiratory metabolism

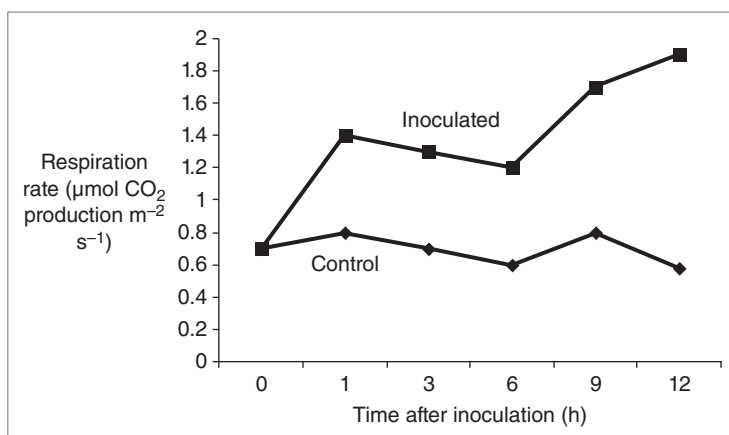


Fig. 4.5 Respiration rate in the incompatible interaction between tobacco leaves and the pathogen *Phytophthora nicotianae*. Adapted from Scharte *et al.*, (2005). Reproduced with permission of John Wiley & Sons.

suggests that *Lr34*-mediated resistance is an extremely energy-intensive process, with the increased demand for energy met by up-regulating glycolysis and the TCA cycle and recruiting alternative metabolic pathways. However, this enhanced respiratory activity is not sustained, and by 7 days after inoculation, none of the respiratory genes was up-regulated (Bolton *et al.*, 2008). The authors suggest that either this level of enhanced activity cannot be maintained for a prolonged period or the metabolism-related genes were suppressed by the pathogen (see Panstruga, 2003). Nevertheless, the loss of the enhanced respiratory activity by 7 days after inoculation might help to explain why the resistance mediated by *Lr34* fails to inhibit the pathogen completely (Bolton *et al.*, 2008).

In some interesting earlier work by Haigh *et al.* (1991), using a range of oat genotypes exhibiting different levels of partial resistance to powdery mildew, little effect on respiration could be detected. Moreover, no differences were observed in the responses of susceptible and resistant genotypes. The authors suggested that perhaps the changes in respiration frequently observed in powdery-mildew-infected plants were delayed or compensated for in oat. It would be useful to conduct a transcriptomic analysis of metabolic genes in these oat genotypes interacting with powdery mildew, to determine whether more subtle changes in respiratory metabolism occur, changes that could not be detected using gas exchange measurements. Nevertheless, this serves to highlight the importance of avoiding generalisations when dealing with plant responses to other organisms.

The success of some pathogens in becoming established in host tissues depends on the production of toxins secreted by the pathogen. These toxins can exert profound changes in host metabolism. One example of a toxin-producing fungus is *Helminthosporium victoriae*, the cause of foot and root rot and leaf blight in oat. This fungus produces a host-specific toxin called victorin (or HV-toxin), which, similarly to *H. victoriae*, is specific for certain oat cultivars. This means that victorin only affects those cultivars that are susceptible to *H. victoriae*. A striking secondary effect of victorin is increased respiration of host tissues, which is proportional to the concentration applied (Fig. 4.6; Krupka, 1958). This action of victorin appears to

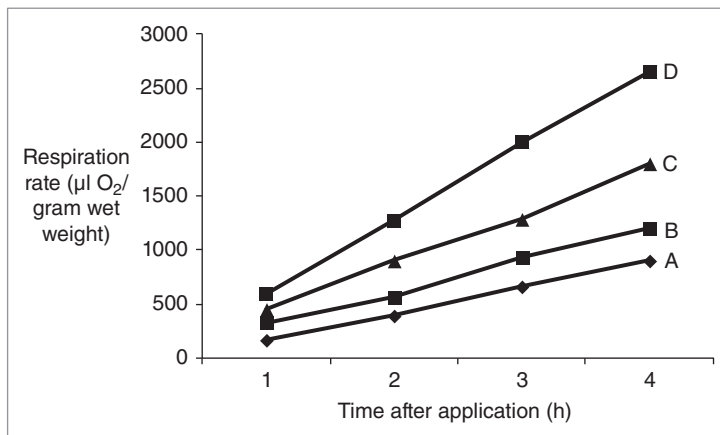


Fig. 4.6 Respiration in susceptible oat tissue treated with increasing concentrations of the toxin, victorin. A=control; B=toxin diluted 1×10^{-4} ; C=toxin diluted 1×10^{-3} ; D=toxin diluted 2.5×10^{-2} . Adapted from Krupka (1958). Reproduced with permission of AAAS.

be indirect, because the ability of mitochondria from sensitive tissues to take up oxygen is not affected by the toxin (Wheeler & Hanchey, 1966).

4.2.2 Effects of bacterial pathogens

As with fungal pathogens, interactions of plants with pathogenic bacteria can result in large increases in respiration. For example, Fischer (1981) examined respiration in compatible and incompatible interactions between pepper fruit tissue and *Xanthomonas vesicatoria*. Respiration rate increased rapidly in resistant host tissue inoculated with the pathogen but did not start to increase in the susceptible host until some 30 hours after inoculation. A similar trend was observed in *Arabidopsis thaliana* interacting with avirulent or virulent strains of *Pseudomonas syringae* pv. *tomato* (*Pst*). In the incompatible interaction between *A. thaliana* and the avirulent strain of *Pst*, respiration rate had increased substantially above controls by 24 hours after inoculation, while in the compatible interaction, respiration rate did not start increasing until 24 hours after inoculation, reaching a peak at 40 hours after inoculation (Fig. 4.7; Simons *et al.*, 1999). There were also changes in activity of the AOX pathway in these interactions, and indeed, the rates of SHAM-sensitive oxygen uptake (SHAM – salicylhydroxamic acid, an inhibitor of AOX) followed the same patterns observed for total respiration, with a rapid increase in alternative respiration in the incompatible interaction and a delayed increase in the compatible interaction (Fig. 4.8). This increased engagement of the AOX pathway was confirmed by greatly increased AOX transcript levels (Fig. 4.9). Interestingly, these workers found that the pyruvate levels in leaves inoculated with avirulent *Pst* were greatly increased, suggesting that the mitochondria were flooded with respiratory substrate. As described earlier in this chapter, these conditions are likely to favour full operation of the AOX pathway in cells of infected leaves. Using *Arabidopsis* mutants unable to accumulate salicylic acid, or to induce systemic acquired resistance, the researchers obtained results suggesting that the increased respiration in these interactions between *A. thaliana* and *Pst* was associated primarily with symptom expression rather than resistance reactions (Simons *et al.*, 1999).

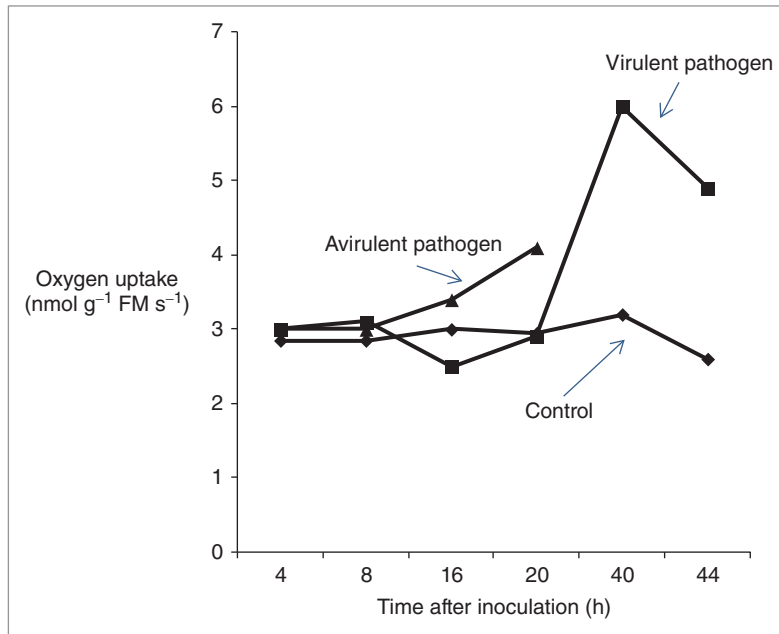


Fig. 4.7 Respiration rates of wild-type *Arabidopsis* leaves infiltrated with avirulent (incompatible interaction) or virulent (compatible interaction) strains of *Pseudomonas syringae* pv. *tomato*. Adapted from Simons *et al.* (1999). Reproduced with permission of American Society of Plant Biologists.

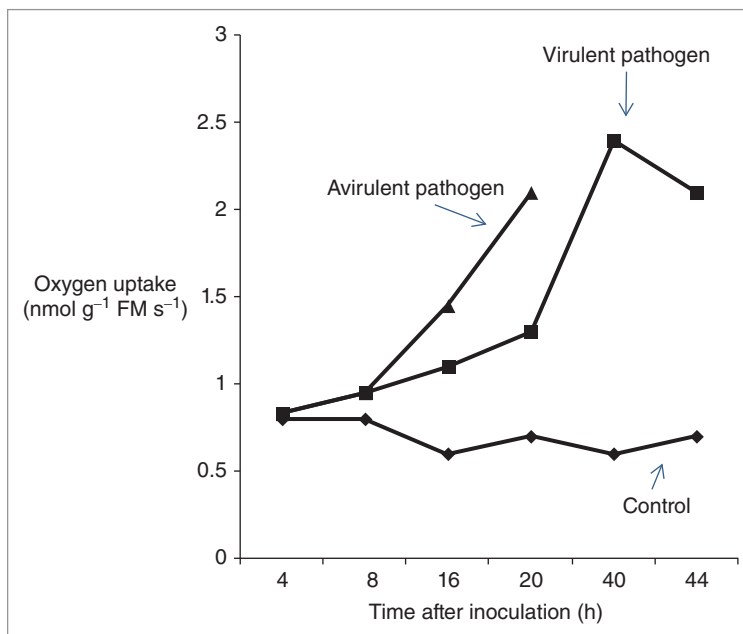


Fig. 4.8 Rates of SHAM-sensitive respiration of wild-type *Arabidopsis* leaves infiltrated with avirulent (incompatible interaction) or virulent (compatible interaction) strains of *Pseudomonas syringae* pv. *tomato*. Adapted from Simons *et al.* (1999). Reproduced with permission of American Society of Plant Biologists.

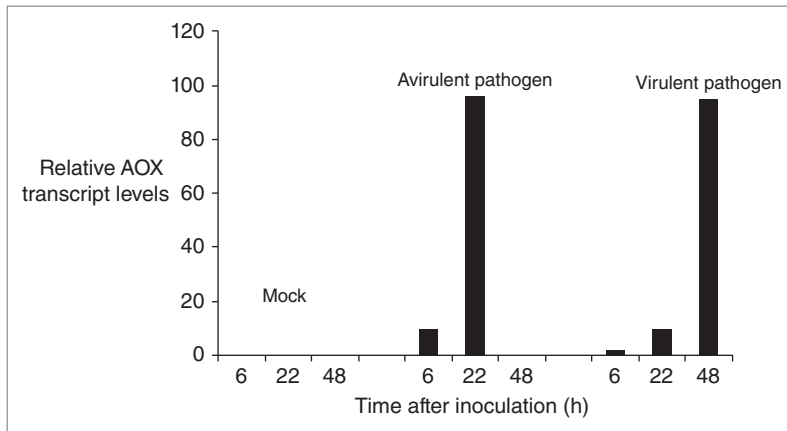


Fig. 4.9 AOX transcript levels in wild-type *Arabidopsis* leaves infiltrated with avirulent (incompatible interaction) or virulent (compatible interaction) strains of *Pseudomonas syringae* pv. *tomato*. Adapted from Simons *et al.* (1999). Reproduced with permission of American Society of Plant Biologists.

4.2.3 Effects of viruses

Goodman *et al.* (1986) highlight the importance of exercising caution when interpreting changes in respiration in plant–virus interactions. Important considerations include the fact that virus can be confined to the area of local lesions, but might also replicate systemically, in non-inoculated tissue. Indeed, changes in respiration of systemically infected tissue might differ substantially from changes occurring in necrotic lesions. In leaves on non-hypersensitive hosts, inoculation with virus can lead to a small increase in respiration, although decreased respiration rates have also been reported (e.g. Owen, 1958; Jensen, 1968). A more complex pattern was obtained by Takahashi and Hirai (1964), working on tobacco inoculated with the tobacco mosaic virus (TMV). They attempted to reduce interference from uninfected cells by inoculating the lower epidermis, stripping it off at various times after inoculation and comparing the respiration rate in epidermal cells to that in uninoculated controls. They found that compared to controls, respiration rate of infected tissue increased rapidly but began to decline 2 days after inoculation, falling to below control levels with time (Fig. 4.10). Moreover, this fall in respiration coincided with accumulation of virus in the infected tissue (Fig. 4.10; Takahashi & Hirai, 1964). However, this pattern of increased respiration early in the interaction has not been widely reported. Indeed, in a number of systems, including tobacco infected with tobacco etch virus or potato virus X (PVX), respiration rate did not increase until symptoms appeared (Owen, 1957, 1958; Dwurazna & Weintraub, 1969). According to Goodman *et al.* (1986), this might reflect the fact that whole leaves were used in these studies, with cells infected asynchronously, with the result that changes in respiration only become detectable later in infection.

In interactions where the host responds hypersensitively, there tends to be a pronounced rise in respiration, with increases detected several hours before the appearance of lesions in a variety of host–virus combinations (e.g. Sunderland & Merrett, 1965; Chant, 1967). This is not a universal phenomenon, however, because other workers have failed to detect an increase in respiration until symptoms appeared (Yamaguchi & Hirai, 1959). A number of workers have

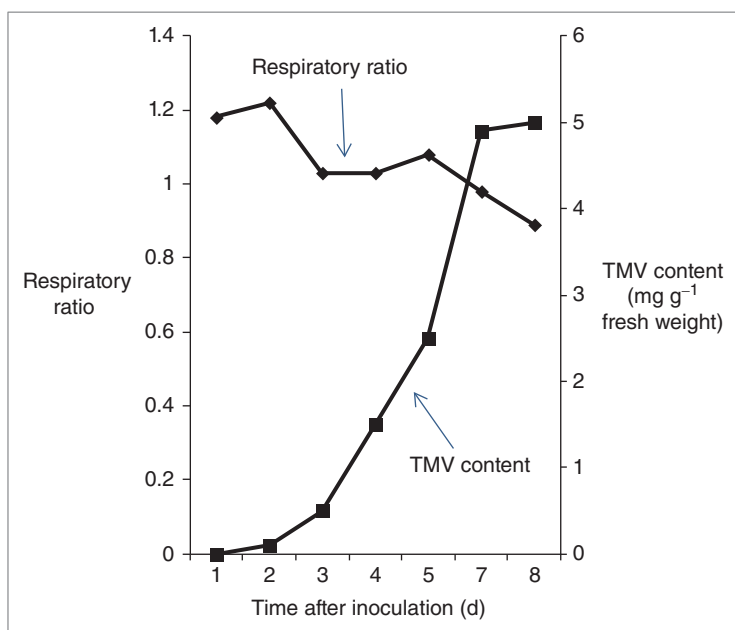


Fig. 4.10 The ratios of respiratory rates between TMV-inoculated and uninoculated tobacco leaf epidermis at different times after inoculation, and the TMV content of inoculated epidermis. Takahashi and Hirai (1964). Reproduced with permission of John Wiley & Sons.

suggested that the increase in respiration in hypersensitive hosts is related to symptom severity (i.e. necrosis) rather than virus replication (Yamaguchi & Hirai, 1959; Parish *et al.*, 1965). In leaves responding hypersensitively to virus infection, at least part of the increased respiration is due to stimulation of the OPP pathway (e.g. Solymosy & Farkas, 1962, 1963).

Work on the physiological changes associated with infection of cotyledons of marrow (*Cucurbita pepo*) with cucumber mosaic virus (CMV) was reported in a series of articles by Técsi *et al.* (1994a,b,c). The first symptoms of CMV infection are chlorotic lesions that appear about 4 days after inoculation. These lesions are not homogeneous but rather comprise circular zones exhibiting diverse physiology. Thus, going from the outer edge of the lesion, four zones can be distinguished: (1) a zone (i) of infected cells that do not accumulate starch, (2) a zone (ii) consisting of cells with increased photosynthetic activity, with accumulation of starch, (3) a zone (iii) of cells largely devoid of starch, with low photosynthetic activity and (4) a zone (iv) of cells surrounding the initial infection point, which retains high photosynthetic activity and a high starch content for the initial 4–6 days after infection. As the course of infection progresses and the lesion expands, after 10 days, the inner region comprising iii and iv becomes a dominant feature, resulting in generalised chlorosis. When whole cotyledons were examined, infection with CMV led to a substantial increase in respiration, together with increased capacities of the OPP pathway, glycolysis, mitochondrial electron transport, the TCA cycle and starch degradation (Técsi *et al.*, 1994b). However, when the metabolic changes occurring within the different zones in the lesion were examined, a more complex picture was revealed (Fig. 4.11). At the periphery of the lesion (i), there was virus replication and synthesis of virus

protein. This created a strong sink demand and was associated with increased photosynthesis, starch accumulation, and increased activities of anaplerotic enzymes (e.g. NADP-dependent malic enzyme – probably important in C_3 plants in providing pyruvate and NADPH for biosynthetic reactions and respiration). Within the lesion (zones ii, iii and iv), once virus synthesis had declined, photosynthesis was reduced, starch mobilised, and metabolism shifted towards glycolysis and mitochondrial respiration (Fig. 4.11). These data suggest that cells in which

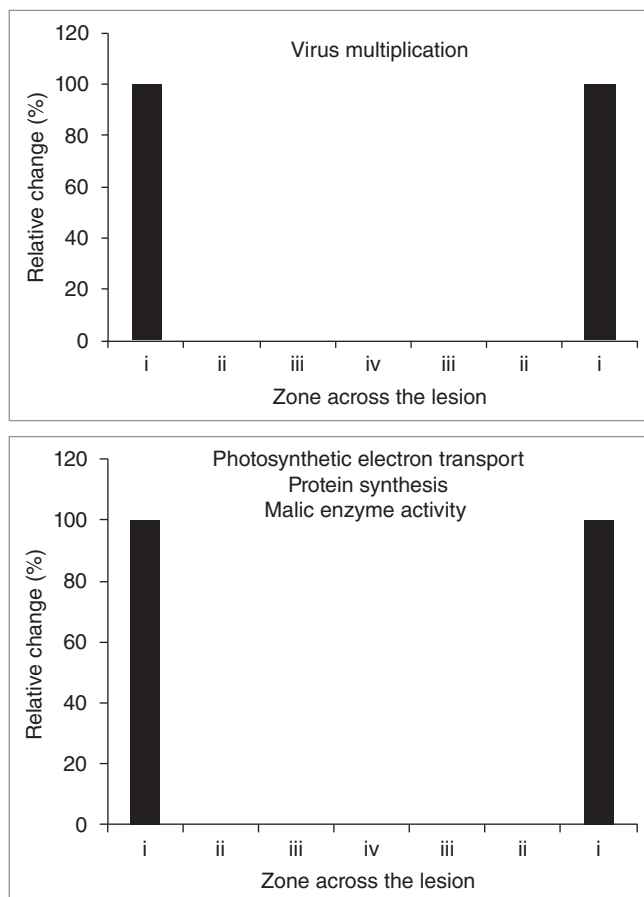


Fig. 4.11 Relative changes in a series of biochemical responses to infection across the lesion on cotyledons of marrow infected with cucumber mosaic virus. The circular zones of cells across the lesion are indicated as i–iv. At the sites of virus replication, protein synthesis and activity of NADP-dependent malic enzyme are stimulated. The rate of photosynthetic electron transport is also stimulated in the region during the induction phase of photosynthesis but is lower in the centre of the lesion even during steady-state photosynthesis. Inside the lesion, starch accumulates and other biosynthetic processes are elevated, as indicated by the increased activities of enzymes of the OPP pathway. At the same time, the activities of Calvin cycle enzymes decline. Finally, starch degradation, glycolysis and respiration are activated, and chlorosis starts developing in the centre of the lesion. Técsi *et al.* (1996). Reproduced with permission of American Society of Plant Biologists.

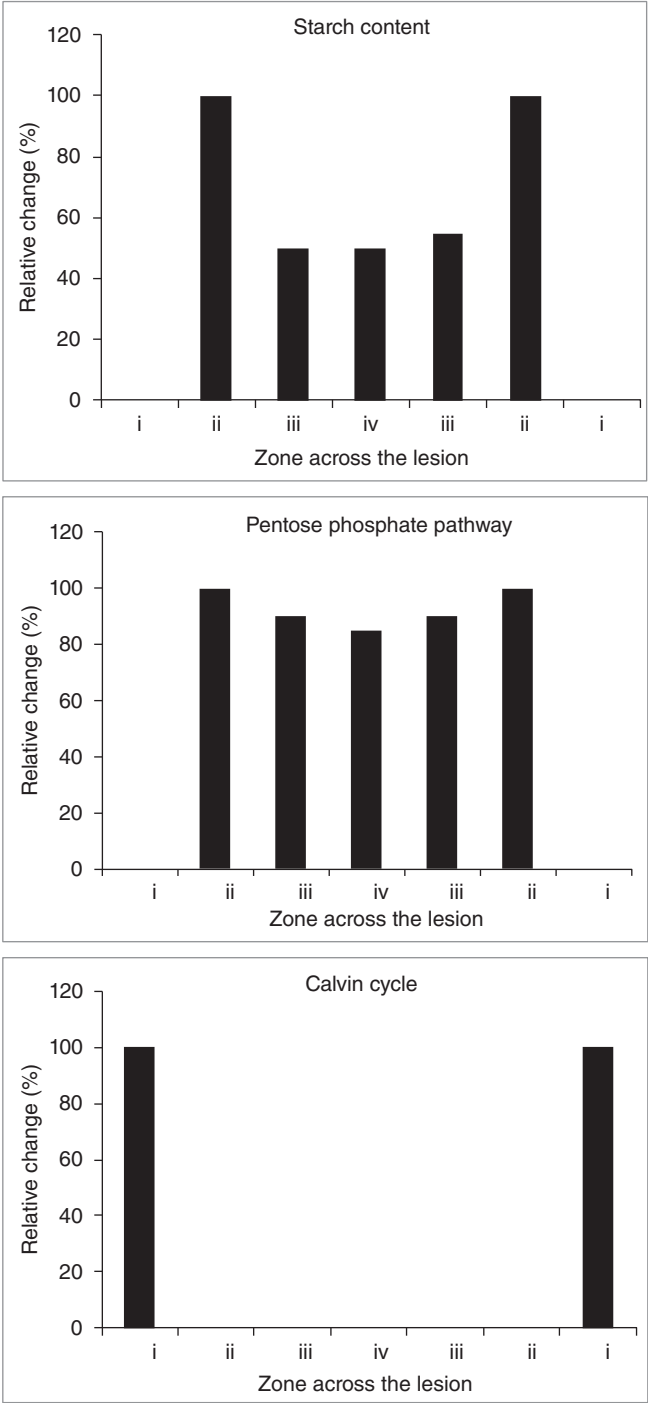


Fig. 4.11 (continued).

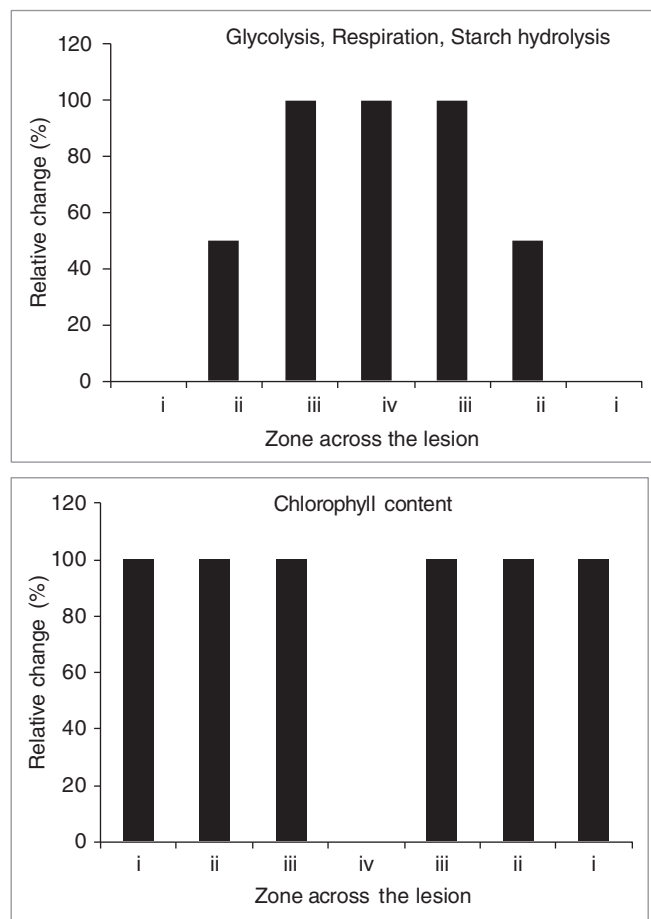


Fig. 4.11 (continued).

the virus is replicating constitute a powerful sink, with greatly enhanced biosynthetic activity, reflected in the increased activities of enzymes of the OPP pathway and of NADP-malic enzyme at the periphery of the lesion (Técsi *et al.*, 1996).

Increases in respiration, including increased activities of glycolysis, the TCA cycle, the pentose phosphate pathway (PPP) and mitochondrial electron transport, are thought to be induced after pathogen attack in order to generate energy for defence responses (Bolton, 2009). However, this is not always the case. For example, in tomato infected with *Pepino Mosaic Virus* (PepMV), although defences were induced, there was a strong repression of the TCA cycle (Hanssen *et al.*, 2011). This suggests that, rather than the plant freeing up resources for defence, the virus modulates the host by repressing all metabolic processes that are not required for virus replication (Hanssen *et al.*, 2011) (Box 4.1).

Box 4.1 The alternative oxidase and virus resistance

A by-product of activity of the electron transport chain in mitochondria is the generation of ROS (Noctor *et al.*, 2007). Alterations to this ROS pool can function in intracellular signal transduction, ultimately affecting the pattern of nuclear gene expression (Noctor *et al.*, 2007). Such signalling can be influenced by the AOX. Functions of the AOX pathway include negative regulation of ROS production in mitochondria and maintenance of primary metabolism under stress (Moore *et al.*, 2002; Pasqualini *et al.*, 2007).

Mitochondria have been implicated in local and systemic plant defences against pathogens. As we saw previously, AOX is induced in *Arabidopsis* interacting with *Pst* (Simons *et al.*, 1999), while treatment with the elicitor harpin leads to increased expression of the *AOX1a* gene in suspension cells of *Arabidopsis* (Krause & Durner, 2004). AOX is also induced in plant–virus interactions, and indeed, several lines of evidence support the idea that the AOX-dependent signal transduction pathway plays a role in mediating virus localisation and limiting host necrosis (Chivasa & Carr, 1998; Love *et al.*, 2005; Király *et al.*, 2008). Using a compatible interaction between tomato and TMV, Fu *et al.* (2010) showed that cyanide-resistant (alternative) respiration and expression of AOX genes were increased within 12 hours in upper uninoculated leaves after inoculation of the lower leaves with the virus. Interestingly, levels of nitric oxide (NO), which is known to be involved in plant responses to environmental cues, including pathogen infection, were also increased in upper leaves with 12 hours. Furthermore, this TMV-induced induction of AOX was enhanced by the application of NO and blocked when a NO scavenger was used. Not only did the NO scavenger block the induction of AOX, but also it increased susceptibility to TMV. The data obtained by Fu *et al.* (2010) led them to suggest that TMV-induced generation of NO mediates AOX induction, which then induces the activity of the AOX pathway, triggering systemic basal defence against TMV.

Further support for the involvement of the AOX pathway in basal resistance to virus infection was provided by Lee *et al.* (2011). They generated transgenic plants of *Nicotiana benthamiana* in which the capacity of the AOX pathway was either increased by constitutive expression of AOX (*Aox* transgenic plants) or decreased by expression of a dominant negative mutant protein (AOX-E). Modifying the AOX pathway in this way had dramatic effects on disease symptoms in plants infected with PVX and on accumulation of PVX virus in directly inoculated and systemically infected leaves (Fig. 4A). Thus, at 14 days after inoculation, significantly less virus had accumulated in plants expressing AOX-E than in those expressing AOX (i.e. *Aox* transgenic plants). Salicylic acid (SA) is known to induce resistance in plants, and indeed, it induces resistance in *N. benthamiana* to PVX. However, this SA-induced resistance to PVX was compromised in *Aox* transgenic plants, whereas plants expressing AOX-E exhibited enhanced SA-induced resistance to PVX (Fig. 4B). These data led the authors to conclude that AOX-regulated mechanisms not only play a role in SA-induced resistance, but also contribute to basal resistance against certain viruses, for example PVX (Lee *et al.*, 2011). Different results, although still supporting the hypothesis that the AOX pathway is involved with resistance to viruses, are the work of Lee *et al.* (2011). In experiments using transgenic tomato and petunia with

elevated levels of AOX expression, they found that such plants exhibited enhanced resistance to tomato spotted wilt virus (Ma *et al.*, 2011). As the number of plant species and viruses studied increases, it is becoming clear that different host species can use different mechanisms to resist virus infection (Mayers *et al.*, 2005). It would appear therefore that one size does not fit all!

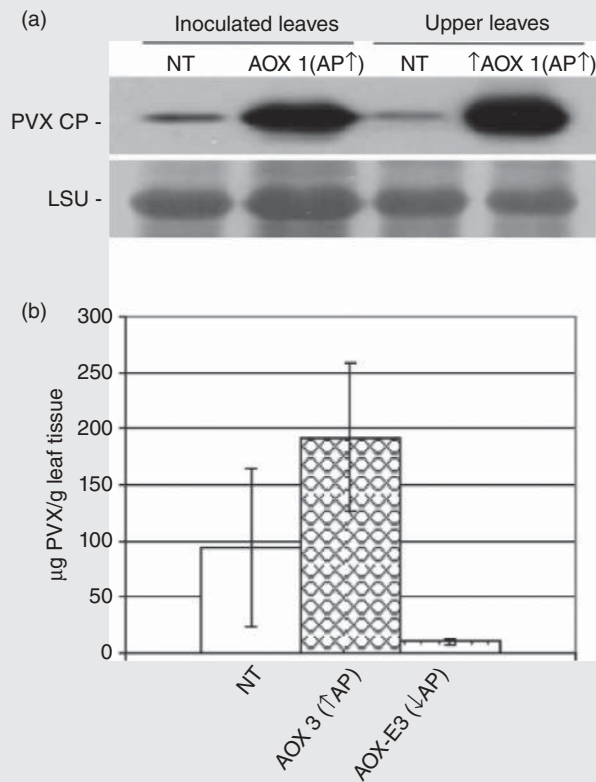


Fig. 4A Susceptibility of plants to PVX accumulation in directly inoculated and systemically infected leaves is altered by modification of alternative respiratory pathway (AP) capacity. (a) Semi-quantitative analysis of virus accumulation in PVX-inoculated non-transgenic and Aox-transgenic plants by immunoblot analysis using anti-PVX coat protein (CP). This showed that virus accumulation was higher in directly inoculated and systemically infected leaves of plants with increased AP capacity than in corresponding tissues of non-transgenic plants. Lee *et al.* (2011). © Lee *et al.*; licensee BioMed Central Ltd/CC BY 2.0. (b) Quantitative analysis (enzyme-linked immunosorbent assay) of PVX coat protein accumulation in non-transgenic (NT) plants and plants belonging to transgenic lines with increased (↑) or decreased (↓) AP capacity. Systemically-infected (i.e. upper, not directly inoculated) leaf tissue was harvested at 14 days after inoculation. Tissue from the fourth leaf above the inoculated leaf was taken in each case. There is a significant difference in PVX accumulation in plants with decreased AP capacity compared to those with increased AP capacity. From Lee *et al.* (2011) <http://www.biomedcentral.com/1471-2229/11/41>.

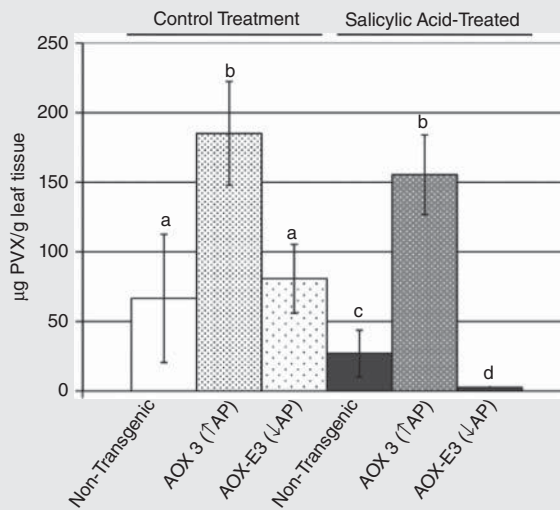


Fig. 4B Effects of altering alternative respiratory pathway capacity on SA-induced resistance to PVX. Plants were pre-treated by spraying with a solution of SA or a control solution for 4 days before inoculation with PVX. Samples from the fourth leaf above the directly PVX-inoculated leaves of plants were harvested at 28 days after inoculation and processed for an enzyme-linked immunosorbent assay using anti-PVX coat protein serum. Accumulation of virus was significantly different between non-transgenic and AOX 3 Aox-transgenic plants and between SA-treated and SA-treated AOX3 Aox-transgenic plants. Mean titre values labelled with the same lower case letter were not significantly different from each other (*t*-test, $p > 0.05$). Lee *et al.* (2011). © Lee *et al.*; licensee BioMed Central Ltd/CC BY 2.0.

4.2.4 Effects of insect herbivores

Insect herbivory can result in increased respiration rates in damaged leaves. For example, damage caused by feeding of first and fourth instars of *Trichoplusia ni* led to significantly increased rates of respiration in leaves of *A. thaliana* (Tang *et al.*, 2006). These authors speculated that the enhanced respiration might have been a wound response or been related to accelerated production of defensive chemicals. Indeed, earlier research by Zangerl *et al.* (1997) found that mechanical damage to leaves of wild parsnip increased respiration in those leaves, and furthermore, significant increases in respiration were evident from just 2 hours after damage was inflicted (Fig. 4.12). Interestingly, this increased respiration was associated with an increased content of defensive furanocoumarins in damaged leaves 2 hours after treatment (Fig. 4.13), and importantly, variation among plants in damage-induced furanocoumarin production was correlated with damage-induced increases in respiration. Zangerl *et al.* (1997) calculated that the energetic cost of furanocoumarin production ($12.6 \mu\text{g glucose cm}^{-2}$) accounted for all of the increase in respiration ($12.0 \mu\text{g glucose cm}^{-2}$), highlighting the tight linkage between furanocoumarin synthesis and damage-induced rates of respiration. Although photosynthesis was reduced in damaged leaves, the effect was short-lived, making it likely that the growth reductions observed were the result of the enhanced synthesis of furanocoumarins. Clearly, defence comes at a price!

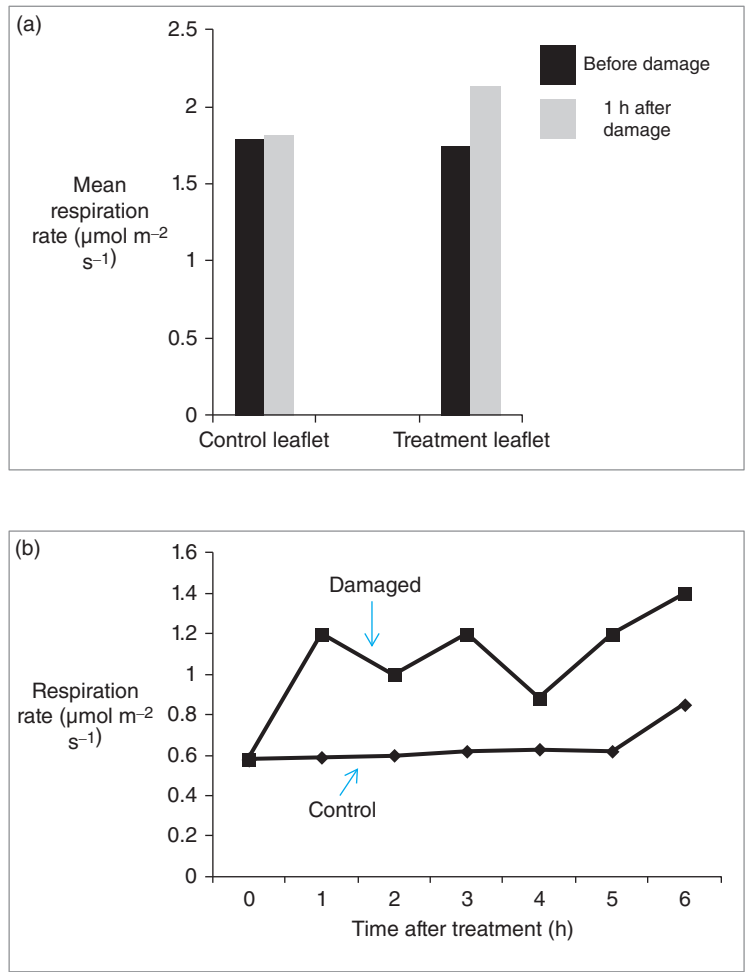


Fig. 4.12 (a) Localisation of damage-induced respiration in leaflets of wild parsnip. Respiration rate in the leaflet 1 hour after damage is significantly different from respiration rate in the control leaflet. Data from Zangerl *et al.* (1997). (b) Respiration of damaged and intact leaflets of a wild parsnip plant at hourly intervals following treatment. The damaged leaflet had a significantly greater respiration rate than the intact leaflet at all times after treatment, except at time 0. Data from Zangerl *et al.* (1997).

4.2.5 Effects of parasitic plants

Parasitic plants appear to have little impact on rates of respiration of their hosts. Thus, infection of *Mikania micrantha* by the holoparasite *Cuscuta campestris* had little effect on host respiration (Shen *et al.*, 2007), with similar results reported for infection of cowpea by the hemiparasitic *Striga gesnerioides* (Hibberd *et al.*, 1996). Interestingly, rates of respiration in *Striga* itself are very high, and coupled with its low photosynthetic rates the parasite is largely reliant on its host for assimilate (Press *et al.*, 1987).

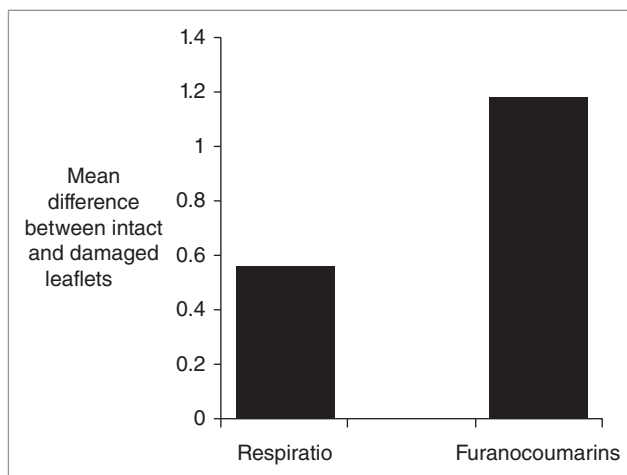


Fig. 4.13 Mean difference in respiration rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and furanocoumarin concentration ($\mu\text{g mg}^{-1}$) between intact and damaged leaflets of wild parsnip 2 hours after treatment. On average, furanocoumarin concentration increased $1.18 \mu\text{g mg}^{-1}$ and respiration rate increased $0.56 \mu\text{mol m}^{-2} \text{s}^{-1}$. Data from Zangerl *et al.* (1997).

4.3 PHOTORESPIRATION IN ATTACKED PLANTS

As we saw at the beginning of this chapter, Rubisco can catalyse the oxygenation of RuBP. This leads to the formation of glycolate 2-phosphate, which is then metabolised via the glycolate pathway (Fig. 4.14). This is known as the photorespiratory pathway and is compartmentalised across three organelles: chloroplasts, peroxisomes and mitochondria. Glycolate 2-phosphate is hydrolysed to glycolate in the chloroplast, which in turn is oxidised in the peroxisome to glyoxylate. This glyoxylate is then transaminated to yield the amino acid glycine, which is transported to the mitochondrion. In this case, it is converted to serine, with the release of CO_2 and NH_3 , the serine then returning to the peroxisome, where it is converted to hydroxypyruvate and then to glycerate. The glycerate is transported to the chloroplast, where it is phosphorylated, before entering the Calvin cycle as glycerate 3-phosphate (Fig. 4.14). It has been suggested that photorespiration is important for maintaining electron flow in order to prevent photoinhibition occurring under conditions of stress (Wingler *et al.*, 2000). Photoinhibition can occur when the photochemical processes of photosynthesis have insufficient ADP or NADP to allow the continued flow of energy through the electron transport system (Huner *et al.*, 1998). As molecules within the electron transport chain become reduced, the flow of electrons becomes blocked. Because light energy continues to be absorbed, electrons in photosystem II become highly excited and the excess energy damages the D1 protein. Photoinhibition is usually temporary, but if other molecules in the photosystem are damaged, there can be bleaching of chlorophyll, resulting in photo-oxidation. The latter can occur in plants exposed to abiotic stress, for example salinity and drought.

Photorespiration increases in plants exposed to abiotic stresses, such as salt stress, but what happens in plants exposed to biotic stress? Compared to other areas of plant metabolism, effects of the biotic environment on photorespiratory activity in the host have received

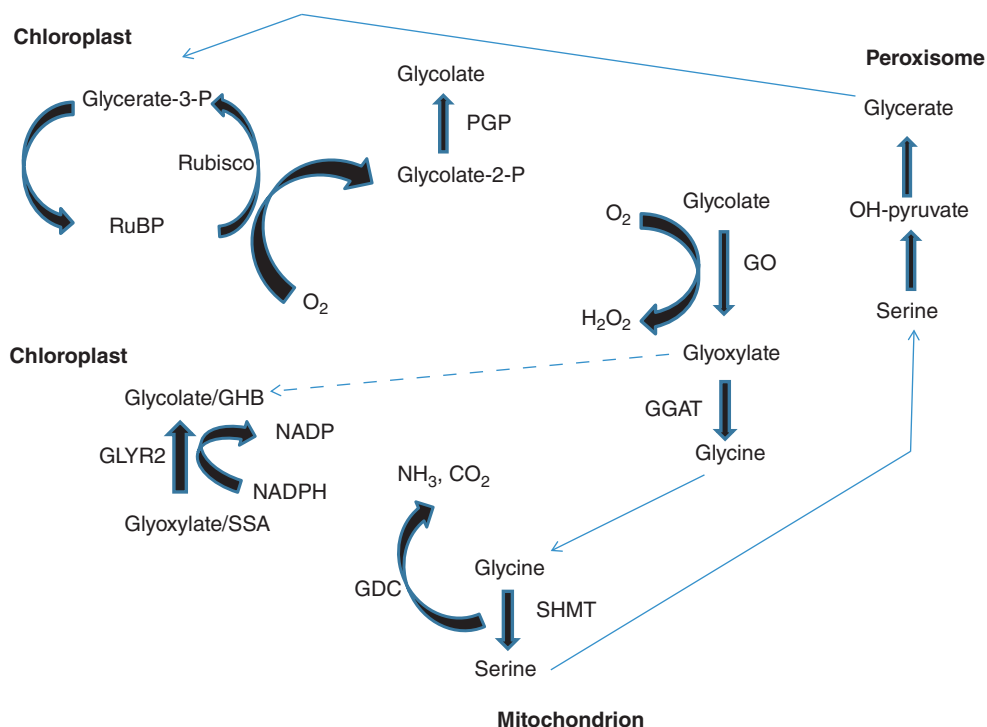


Fig. 4.14 Outline of the photorespiratory cycle in plants. The dotted line represents a potential diffusion pathway. PGP, phosphoglycolate phosphatase; GO, glycolate oxidase; GDC, glycine decarboxylase; SHMT, serine hydroxymethyltransferase; GGAT, glutamate:glyoxylate aminotransferase; SSA, succinic semialdehyde; GHB, γ -hydroxybutyrate; GLYR2, glyoxylate reductase. Allan *et al.* (2009). Reproduced with permission of the Biochemical Society.

relatively little attention. In the following examples, I will concentrate on plants interacting with pathogens.

In a compatible interaction between oak and the powdery mildew, *Microsphaera alphioides*, the rate of photorespiration was reduced in infected plants, declining steadily with time after inoculation (Fig. 4.15; Hewitt & Ayres, 1975). This reduction in photorespiration was subsequently attributed, at least in part, to the reduction in activity of glycolate oxidase in infected leaves (Fig. 4.16; Hewitt & Ayres, 1977). In fact, reduced rates of photorespiration in wheat infected with black stem rust and in flax infected with the rust, *Melampsora lini*, were also attributed to inhibition of glycolate oxidase activity (Király & Farkas, 1957; Kakkar, 1966). In the oak–powdery mildew interaction, Hewitt and Ayres (1977) also found that the activity of glyoxylate reductase (see Fig. 4.14) was also reduced. A reduction in the activity of this enzyme could lead to an accumulation of NADPH. As the authors suggested, because the glycolate-glyoxylate cycle may maintain electron flow in the photosystems for ATP production by using excess NADPH, the reduced activity of glyoxylate reductase might reduce photosynthetic efficiency and levels of ATP. Interestingly, additional effects are possible. Thus, reduced activity of glyoxylate reductase could well lead to an accumulation of glyoxylate, which is a reactive aldehyde and is known to inhibit the activation state of Rubisco

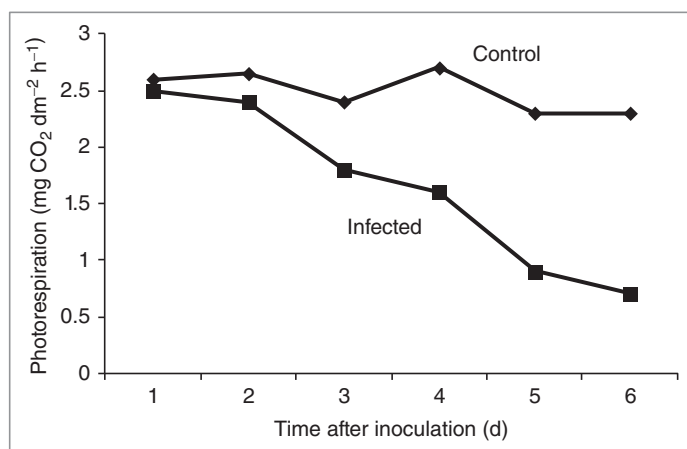


Fig. 4.15 Changes in photorespiration rate of young leaves of oak after infection by powdery mildew, *Microsphaera albidoides*. Photorespiration was measured using the 'post-illumination outburst' method. Hewitt and Ayres (1975). Reproduced with permission of Elsevier.

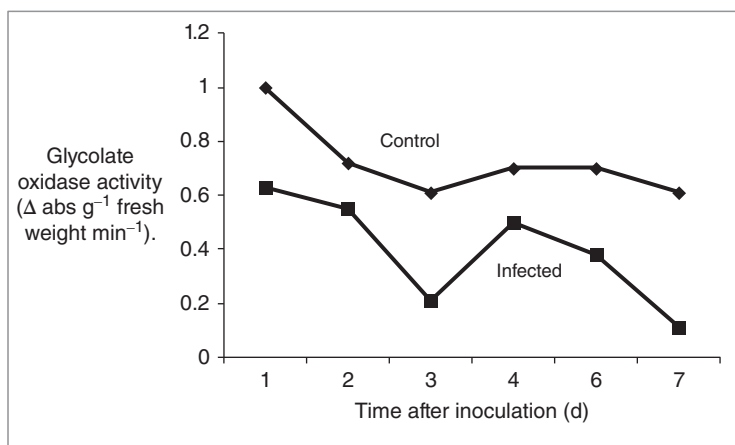


Fig. 4.16 Effect of powdery mildew infection on glycolate oxidase activity in oak leaves. Hewitt and Ayres (1977). Reproduced with permission of John Wiley & Sons.

(Campbell & Ogren, 1990; Hausler *et al.*, 1996). However, in powdery-mildew-infected oak leaves, glyoxylate levels are unlikely to increase, as the activities of both glycolate oxidase and glyoxylate reductase are reduced. Nevertheless, the reduced activity of glycolate oxidase might be important in another way. When glycolate is oxidised to glyoxylate, in the reaction catalysed by glycolate oxidase in the peroxisome, H₂O₂ is released (Fig. 4.14; Foyer & Noctor, 2003). A reduction in glycolate oxidase activity is likely to reduce this H₂O₂ production. Whether such a reduction in H₂O₂, which is known to be involved in defence signalling and pathogen defence (e.g. Kangasjärvi *et al.*, 2012), plays any role in the compatible interaction between oak and powdery mildew is not known.

Peroxisomes have been observed to congregate at the site of fungal invasion, suggesting a role in plant–pathogen interactions (Lipka *et al.*, 2005). Peroxisomal H_2O_2 is notably metabolized by catalases, although ascorbate peroxidases are also associated with peroxisomes (Nyathi and Baker, 2006). Plant lines deficient in catalase have been particularly useful in the analysis of oxidative stress responses (e.g. Takahashi *et al.*, 1997; Chamnongpol *et al.*, 1998; Queval *et al.*, 2007). Under conditions where H_2O_2 produced by photorespiration is high, catalase-deficient plants have been shown to display a marked perturbation of intracellular redox state, measured as glutathione status (e.g. Queval *et al.*, 2007). In tobacco, such redox perturbation has been linked to the activation of certain pathogen-associated processes such as lesion formation, SA accumulation and induction of pathogenesis-related (PR) genes (Takahashi *et al.*, 1997; Chamnongpol *et al.*, 1998; Mittler *et al.*, 1999).

In 1989, researchers identified a genotype of melon from India (PI) that exhibited resistance to all known pathotypes of the downy mildew parasite *Pseudoperonospora cubensis* (Cohen *et al.*, 1989). Resistance in PI was found to be associated with the presence of a tissue-specific cytoplasmic protein, P45, which was not detected in susceptible melons (Balass *et al.*, 1992). After partial sequencing of P45, two genes were cloned from PI corresponding to the sequenced peptides. These genes were found to encode glyoxylate aminotransferases (Taler *et al.*, 2004). These aminotransferases are peroxisomal enzymes involved in the production of glycine during photorespiration. Overexpression of these two genes in susceptible melons led to increased resistance to *P. cubensis*. Glyoxylate aminotransferase activity in leaf extracts was highly correlated with resistance to the pathogen in 13 transgenic lines of melon overexpressing the two genes (Taler *et al.*, 2004). Interestingly, these workers also found that resistance to *P. cubensis* was correlated with higher levels of glycolate oxidase activity, leading them to the conclusion that there is a considerably greater flux through the photorespiratory pathway in resistant plants compared to susceptible melons. Taler *et al.* (2004) suggested that the H_2O_2 produced as a result of this photorespiratory activity is responsible for the resistance observed in the PI-resistant melon genotype. Later work from this laboratory demonstrated that susceptibility of the melon variety Hemed to downy mildew was the result of down regulation of the two glyoxylate aminotransferase genes (Benjamin *et al.*, 2009).

4.4 CONCLUSION

When a plant is attacked, metabolic priorities change. In plants able to mount an effective resistance response, defences need to be mobilised rapidly; in many cases, the defences need to be made *de novo*. All of this requires energy, reductant and precursor molecules, which, if diverted towards defence, will not be available for other plant processes (e.g. growth and reproduction). Such costs are known as allocation costs (Heil & Baldwin, 2002). Photosynthetic rates are usually sufficient to provide an adequate supply of carbon substrates for biosynthetic reactions such as synthesis of terpenes, but, because nitrogen uptake by plants is limited, synthesis of nitrogen-containing compounds such as alkaloids can compete with protein synthesis for precursors (Harborne, 1993). It has been estimated that terpenoids are less expensive to produce than alkaloids (2.6 g of photosynthetically produced carbon per gram of secondary metabolite for terpenoids, compared to 5 g for alkaloids) (Gulmon & Mooney, 1986). Since defence is so expensive, plants are faced with the dilemma of concentrating valuable resources on growth or on defence (Herms & Mattson, 1992). It is possible that the allocation costs associated with

defence might maintain genetic variation within plant populations by preventing alleles that code for high levels of defence from becoming fixed. Costs of resistance have been demonstrated, as with *Brassica* genotypes varying in myrosinase activity. Genotypes exhibiting high myrosinase activity were more resistant to herbivory than low myrosinase genotypes, but at a price, because seed production was lower in these plants (Mitchell-Olds *et al.*, 1996). Yield penalties have been associated with the increased respiration in barley varieties expressing resistance to powdery mildew (Smedegaard-Petersen & Stolen, 1981), and as described previously, synthesis of defensive compounds in wild parsnip was shown to be tightly linked to increased rates of respiration induced by damage (Zangerl *et al.*, 1997).

However, as we have seen previously, increased respiration is not always associated with defence, and, as with interactions between *Arabidopsis* and *Pst*, there is evidence that increased respiration can be associated with symptom expression (Simons *et al.*, 1999). In compatible interactions between plants and pathogens, where defences are not activated until later in the infection process, rates of respiration increase and probably reflect attempts by the host to mobilise resources, for example to deal with damage or to cope with increased demand.

This book examines the effects of attack on host physiology in discrete sections, examining photosynthesis, respiration, carbohydrate metabolism, and so on, separately. Although this approach was adopted for good reasons, allowing for detailed examination of information in the different areas, in reality, all of these different processes are closely linked in the plant dealing with an attack. So, although we have dealt with photosynthesis and respiration in separate chapters, in order to better understand what really happens in the attacked plant, these processes need to be considered together with changes in carbohydrate metabolism and partitioning. The latter aspect will be tackled in the next chapter, where, towards the end, we will try to piece together the complex and amazing metabolic interactions that shape the outcome of interactions between a host and its attacker.

RECOMMENDED READING

- Bolton MD, 2009. Primary metabolism and plant defense – fuel for the fire. *Molecular Plant-Microbe Interactions* **22**, 487–497.
- Hermes DA, Mattson WJ, 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* **67**, 283–335.
- Kangasjärvi S, Neukermans J, Li S, Aro E-M, Noctor G, 2012. Photosynthesis, photorespiration, and light signalling in defence responses. *Journal of Experimental Botany* **63**, 1619–1636.
- Moore AL, Albury MS, Crighton PG, Affourtit C, 2002. Function of the alternative oxidase: is it still a scavenger? *Trends in Plant Science* **7**, 478–481.

REFERENCES

- Allan WL, Clark SM, Hoover GJ, Shelp BJ, 2009. Role of plant glyoxylate reductases during stress: a hypothesis. *Biochemical Journal* **423**, 15–22.
- Balass M, Cohen Y, Bar-Joseph M, 1992. Identification of a constitutive 45 kD soluble protein associated with resistance to downy mildew in muskmelon (*Cucumis melo* L.) line PI 124111F. *Physiological and Molecular Plant Pathology* **41**, 387–396.
- Benjamin I, Kenigsbuch D, Galperin M, Abrameto JA, Cohen Y, 2009. Cisgenic melons over expressing glyoxylate aminotransferase are resistant to downy mildew. *European Journal of Plant Pathology* **125**, 355–365.
- Bolton MD, Kolmer JA, Xu WW, Garvin DF, 2008. Lr34-Mediated Leaf Rust Resistance in Wheat: Transcript Profiling Reveals a High Energetic Demand Supported by Transient Recruitment of Multiple Metabolic Pathways. *Molecular Plant-Microbe Interactions* **21**, 1515–1527.

- Bolton MD, 2009. Primary metabolism and plant defense – fuel for the fire. *Molecular Plant-Microbe Interactions* **22**, 487–497.
- Bushnell WR, Allen PJ, 1962. Respiratory changes in barley leaves produced by single colonies of powdery mildew. *Plant Physiology* **37**, 751–758.
- Campbell WJ, Ogren WL, 1990. Glyoxylate inhibition of ribulose biphosphate carboxylase/oxygenase activation in intact, lysed and reconstituted chloroplasts. *Photosynthesis Research* **23**, 257–268.
- Chant SR, 1967. Respiration rates and peroxidase activity in virus infected *Phaseolus vulgaris*. *Experientia* **23**, 676–677.
- Chamnongpol S, Willekens H, Moeder W, Langebartels C, Sandermann H Jr., Van Montagu M, Inzé D, Van Camp W, 1998. Defense activation and enhanced pathogen tolerance induced by H₂O₂ in transgenic plants. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 5818–5823.
- Chivasa S, Carr JP, 1998. Cyanide restores *N* gene-mediated resistance to tobacco mosaic virus in transgenic tobacco expressing salicylic acid hydroxylase. *Plant Cell* **10**, 1489–1498.
- Clifton R, Millar AH, Whelan J, 2006. Alternative oxidases in Arabidopsis: a comparative analysis of differential expression in the gene family provides new insights into function of non-phosphorylating bypasses. *Biochimica et Biophysica Acta* **1757**, 730–741.
- Cohen Y, Eyal H, Hanania J, Malik Z, 1989. Ultrastructure of *Pseudoperonospora cubensis* in muskmelon genotype susceptible and resistant to downy mildew. *Physiological and Molecular Plant Pathology* **34**, 27–40.
- Dwurazna MM, Weintraub M, 1969. The respiratory pathways of tobacco leaves infected with potato virus X. *Canadian Journal of Botany* **47**, 731–736.
- Farrar JF, Rayns FW, 1987. Respiration of leaves of barley infected with powdery mildew: increased engagement of the alternative oxidase. *New Phytologist* **107**, 119–125.
- Fischer E, 1981. Relation between the transmembrane potential, the energy status, and the ion content in pepper fruit tissue affected by bacterial infection. *PhD dissertation, Department of Plant Pathology, University of Missouri-Columbia*.
- Foyer CH, Noctor G, 2003. Redox sensing and signalling associated with reactive oxygen produced in chloroplasts and mitochondria. *Physiologia Plantarum* **119**, 355–364.
- Fu L-J, Shi K, Gu M, Zhou Y-H, Dong D-K, Liang W-S, Song F-M, Yu J-Q, 2010. Systemic induction and role of mitochondrial alternative oxidase and nitric oxide in a compatible tomato-tobacco mosaic virus interaction. *Molecular Plant Microbe Interactions* **23**, 39–48.
- Goodman RN, Király Z, Wood KR, 1986. *The biochemistry and physiology of plant disease*. Columbia, Missouri: University of Missouri Press.
- Gulmon SL, Mooney HA, 1986. Costs of defense on plant productivity. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge: Cambridge University Press, pp. 681–698.
- Haigh GR, Carver TLW, Gay AP, Farrar JF, 1991. Respiration and photosynthesis in oats exhibiting different levels of partial resistance to *Erysiphe graminis* D.c. ex *Merat* f.sp. *avenae* Marchal. *New Phytologist* **119**, 129–136.
- Hanssen IM, van Esse HP, Ballester A-R, Hogewonig SW, Parra NO, Paeleman A, Lievens B, Bovy AG, Thomma BPHJ, 2011. Differential tomato transcriptomic responses induced by Pepino Mosaic Virus isolates with differential aggressiveness. *Plant Physiology* **156**, 301–318.
- Harborne JB, 1993. *Introduction to ecological biochemistry*. London: Academic Press.
- Hausler RE, Bailey KJ, Lea PJ, Leegood RC, 1996. Control of photosynthesis in barley mutants with reduced activities of glutamine synthetase and glutamate synthase. III. Aspects of glyoxylate metabolism and effects of glyoxylate on the activation state of ribulose-1,5-bisphosphate carboxylase-oxygenase. *Planta* **200**, 388–396.
- Heil M, Baldwin IT, 2002. Fitness costs of induced resistance: emerging experimental support for a slippery concept. *Trends in Plant Science* **7**, 61–67.
- Herns DA, Mattson WJ, 1992. The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* **67**, 283–335.
- Herrmann KM, Weaver LM, 1999. The shikimate pathway. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 473–503.
- Hewitt HG, Ayres PG, 1975. Changes in CO₂ and water vapour exchange rates in leaves of *Quercus robur* infected by *Microsphaera alphitoides* (powdery mildew). *Physiological Plant Pathology* **7**, 127–137.
- Hewitt HG, Ayres PG, 1977. Changes in glycolate oxidase and glyoxylate reductase in *Quercus robur* infected by *Microsphaera alphitoides*. *Physiologia Plantarum* **40**, 31–34.

- Hibberd JM, Quick WP, Press MC, Scholes JD, 1996. The influence of the parasitic angiosperm *Striga gesneriodes* on the growth and photosynthesis of its host, *Vigna unguiculata*. *Journal of Experimental Botany* **47**, 507–512.
- Huner NPA, Öquist G, Sarhan F, 1998. Energy balance and acclimation to light and cold. *Trends in Plant Science* **3**, 224–230.
- Jensen SG, 1968. Photosynthesis, respiration and other physiological relationships in barley infected with barley yellow dwarf virus. *Phytopathology* **58**, 204–208.
- Kakkar RK, 1966. Decline in glycolic acid oxidase activity in flax leaves infected with *Melampsora lini* (Pers.). *Phytopathologische Zeitschrift* **55**, 172–176.
- Kangasjärvi S, Neukermans J, Li S, Aro E-M, Noctor G, 2012. Photosynthesis, photorespiration, and light signalling in defence responses. *Journal of Experimental Botany* **63**, 1619–1636.
- Király L, Hafez YM, Fodor J, Király Z, 2008. Suppression of tobacco mosaic virus-induced hypersensitive-type necrotization in tobacco at high temperature is associated with down-regulation of NADPH oxidase and superoxide and stimulation of dehydroascorbate reductase. *Journal of General Virology* **89**, 799–808.
- Király Z, Farkas GL, 1957. Decrease in glycolic acid oxidase activity in wheat leaves infected with *Puccinia graminis* var. *tritici*. *Phytopathology* **47**, 277–278.
- Kneale J, Farrar JF, 1985. The localisation and frequency of haustoria in colonies of brown rust on barley leaves. *New Phytologist* **101**, 495–505.
- Krause M, Durner J, 2004. Harpin inactivates mitochondria in *Arabidopsis* suspension cells. *Molecular Plant-Microbe Interactions* **17**, 131–139.
- Krupka LR, 1958. Increased ascorbic acid oxidase activity induced by the fungal toxin, victorin. *Science* **128**, 447–448.
- Lee W-S, Fu S-F, Verchot-Lubicz J, Carr JP, 2011. Genetic modification of alternative respiration in *Nicotiana benthamiana* affects basal and salicylic acid induced resistance to potato virus X. *BMC Plant Biology* **11**, 41.
- Lipka V, Dittgen J, Bednarek P, *et al.*, 2005. Pre- and post-invasion defences both contribute to nonhost resistance in *Arabidopsis*. *Science* **310**, 1180–1183.
- Love AJ, Yun BW, Laval V, Loake GJ, Milner JJ, 2005. Cauliflower mosaic virus, a compatible pathogen of *Arabidopsis*, engages three distinct defense-signalling pathways and activates rapid systemic generation of reactive oxygen species. *Plant Physiology* **139**, 935–948.
- Ma H, Song C, Borth W, Sether D, Melzer M, Hu J, 2011. Modified expression of alternative oxidase in transgenic tomato and petunia affects the level of tomato spotted wilt virus resistance. *BMC Biotechnology* **11**, 96.
- Major IT, Nicole M-C, Duplessis S, Séguin A, 2010. Photosynthetic and respiratory changes in leaves of poplar elicited by rust infection. *Photosynthesis Research* **104**, 41–48.
- Mayers CN, Lee KC, Moore CA, Wong SM, Carr JP, 2005. Salicylic acid-induced resistance to cucumber mosaic virus in squash and *Arabidopsis thaliana*: contrasting mechanisms of induction and antiviral action. *Molecular Plant Microbe Interactions* **18**, 428–434.
- McAinsh MR, Ayres PG, Hetherington AM, 1989. The respiration of protoplasts from leaves of barley infected by powdery mildew (*Erysiphe graminis* f.sp. *hordei*). *Plant Science* **64**, 221–230.
- Millerd A, Scott KJ, 1956. Host pathogen relations in powdery mildew of barley. II. Changes in respiratory pattern. *Australian Journal of Biological Sciences* **9**, 37–44.
- Mitchell-Olds T, Siemens D, Pedersen D, 1996. Physiology and costs of resistance to herbivory and disease in Brassica. *Entomologia Experimentalis et Applicata* **80**, 231–237.
- Mittler R, Herr EH, Orvar BL, Van Camp W, Willekens H, Inze D, Ellis BE, 1999. Transgenic tobacco with reduced capacity to detoxify reactive oxygen intermediates are hyper-responsive to pathogen infection. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 14165–14170.
- Moore AL, Albury MS, Crighton PG, Affourtit C, 2002. Function of the alternative oxidase: is it still a scavenger? *Trends in Plant Science* **7**, 478–481.
- Noctor G, De Paepe R, Foyer CH, 2007. Mitochondrial redox biology and homeostasis in plants. *Trends in Plant Science* **12**, 125–134.
- Nyathi Y, Baker A, 2006. Plant peroxisomes as a source of signalling molecules. *Biochimica et Biophysica Acta* **1763**, 1478–1495.
- Öpik H, Rolfe S, 2005. *The physiology of flowering plants*. Cambridge: Cambridge University Press.
- Owen PC, 1957. The effect of infection with tobacco etch virus on the rates of respiration and photosynthesis of tobacco leaves. *Annals of Applied Biology* **45**, 327–331.

- Owen PC, 1958. Photosynthesis and respiration rates of leaves of *Nicotiana glutinosa* infected with tobacco mosaic virus and of *N. tabacum* infected with potato virus X. *Annals of Applied Biology* **46**, 198–204.
- Owera SAP, Farrar JF, Whitbread R, 1981. Growth and photosynthesis in barley infected with brown rust. *Physiological Plant Pathology* **18**, 79–90.
- Panstruga R, 2003. Establishing compatibility between plants and obligate biotrophic pathogens. *Current Opinion in Plant Biology* **6**, 320–326.
- Parish CL, Zaitlin M, Siegel A, 1965. A study of necrotic lesion formation by tobacco mosaic virus. *Virology* **26**, 413–418.
- Pasqualini S, Paolocci F, Borgogni A, Morettini R, Ederli L, 2007. The overexpression of an alternative oxidase gene triggers ozone sensitivity in tobacco plants. *Plant Cell and Environment* **30**, 1545–1556.
- Press MC, Tuohy JM, Stewart GR, 1987. Gas exchange characteristics of the sorghum-*Striga* host parasite association. *Plant Physiology* **85**, 1143–1145.
- Queval G, Issakidis-Bourguet E, Hoeberichts FA, Vandenborgh M, Gakière B, Vanacker H, Miginiac-Maslow M, Van Breusegem F, Noctor G, 2007. Conditional oxidative stress responses in the *Arabidopsis* photorespiratory mutant *cat2* demonstrate that redox state is a key modulator of day-length-dependent gene expression and define photoperiod as a crucial factor in the regulation of H₂O₂ induced cell death. *Plant Journal* **52**, 640–657.
- Roberts AM, Walters DR, 1988. Photosynthesis in discrete regions of leek leaves infected with rust. *New Phytologist* **110**, 371–376.
- Scharte J, Schön H, Weis E, 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell and Environment* **28**, 1421–1435.
- Scholes JD, Farrar JF, 1985. Photosynthesis and chloroplast functioning within individual pustules of *Uromyces muscari* on bluebell leaves. *Physiological and Molecular Plant Pathology* **27**, 387–400.
- Scholes JD, Farrar JF, 1986. Increased rates of photosynthesis in localised regions of a barley leaf infected with brown rust. *New Phytologist* **104**, 601–612.
- Shen H, Hong L, Ye W, Cao, Wang Z, 2007. The influence of the holoparasitic plant *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*. *Journal of Experimental Botany* **58**, 2929–2937.
- Simons BH, Millenaar FF, Mulder L, Van Loon LC, Lambers H, 1999. Enhanced expression and activation of the alternative oxidase during infection of *Arabidopsis* with *Pseudomonas syringae* pv. *tomato*. *Plant Physiology* **120**, 529–538.
- Smedegaard-Petersen V, 1984. The role of respiration and energy generation in diseased and disease-resistant plants. In: Wood RKS, Jellis GJ, eds. *Plant D: infection, damage and loss*. Oxford: Blackwell Scientific Publications, pp. 73–85.
- Smedegaard-Petersen V, Stolen O, 1981. Effect of energy requiring defense reactions on yield and grain quality in a powdery mildew resistant barley cultivar. *Phytopathology* **71**, 396–399.
- Smith AM, Coupland G, Dolan L, Harberd N, Jones J, Martin C, Sablowski R, Amey A, 2010. *Plant biology*. New York: Garland Science.
- Solymosy F, Farkas GL, 1962. Simultaneous activation of pentose phosphate shunt enzymes in a virus infected local lesion host plant. *Nature* **195**, 835.
- Solymosy F, Farkas GL, 1963. Metabolic characteristics at the enzymatic level of tobacco tissues exhibiting localised acquired resistance to viral infection. *Virology* **21**, 210–221.
- Sunderland DW, Merrett MJ, 1965. The respiration of leaves showing necrotic local lesions following infection by tobacco mosaic virus. *Annals of Applied Biology* **56**, 477–484.
- Takahashi H, Chen Z, Du H, Liu Y, Klessig DF, 1997. Development of necrosis and activation of disease resistance in transgenic tobacco plants with severely reduced catalase levels. *Plant Journal* **11**, 993–1005.
- Takahashi T, Hirai T, 1964. Respiratory increase in tobacco leaf epidermis in the early stage of tobacco mosaic virus infection. *Physiologia Plantarum* **17**, 63–70.
- Taler D, Galperin M, Benjamin I, Cohen Y, Kenigsbuch D, 2004. Plant *eR* genes that encode photorespiratory enzymes confer resistance against disease. *The Plant Cell* **16**, 172–184.
- Tang JY, Zielinski RE, Zangerl AR, Crofts AR, Berenbaum MR, De Lucia EH, 2006. The differential effects of herbivory by first and fourth instars of *Trichoplusia ni* (Lepidoptera: Noctuidae) on photosynthesis in *Arabidopsis thaliana*. *Journal of Experimental Botany* **57**, 527–536.
- Técsi LI, Maule AJ, Smith AM, Leegood RC, 1994a. Complex, localised changes in CO₂ assimilation and starch content associated with the susceptible interaction between cucumber mosaic virus and a cucurbit host. *Plant Journal* **5**, 837–847.

- Técsi LI, Maule AJ, Smith AM, Leegood RC, 1994b. Metabolic alterations in cotyledons of *Cucurbita pepo* infected by cucumber mosaic virus. *Journal of Experimental Botany* **45**, 1541–1551.
- Técsi LI, Smith AM, Maule AJ, Leegood RC, 1994c. Physiological studies of viral mosaic infection. In: Walters DR, Scholes JD, Bryson RJ, Paul ND, McRoberts N, eds. *Physiological responses of plants to pathogens. Aspects of applied biology* **42**. Wellesbourne, UK: Association of Applied Biologists, pp. 133–140.
- Técsi LI, Smith AM, Maule AJ, Leegood RC, 1996. A spatial analysis of physiological changes associated with infection of cotyledons of marrow plants with cucumber mosaic virus. *Plant Physiology* **111**, 975–985.
- Van Aken O, Giraud E, Clifton R, Whelan J, 2009. Alternative oxidase: a target and regulator of stress responses. *Physiologia Plantarum* **137**, 354–361.
- Wheeler H, Hanchey PJ, 1966. Respiratory control: loss in mitochondria from diseased plants. *Science* **154**, 1569–1571.
- Wingler A, Lea PJ, Quick WP, Leegood RC, 2000. Photorespiration: metabolic pathways and their role in stress protection. *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* **355**, 1517–1529.
- Yamaguchi A, Hirai T, 1959. The effect of local infection with tobacco mosaic virus on respiration in leaves of *N. glutinosa*. *Phytopathology* **49**, 447–449.
- Zangerl AR, Arntz AM, Berenbaum MR, 1997. Physiological price of an induced chemical defense: photosynthesis, respiration, biosynthesis and growth. *Oecologia* **109**, 433–441.

5 Effects on Carbohydrate Partitioning and Metabolism

5.1 INTRODUCTION

One of the many amazing things about plants is that they are the nutritional foundation for life on the planet. This might not appear particularly remarkable, especially because it means that they face attack by a great many organisms seeking nourishment. A major component of the nourishment being sought is carbohydrate. We have already seen that a common feature of attack is a perturbation in photosynthesis, and indeed, photosynthesis is commonly reduced in plants attacked by pathogens, herbivores or parasitic plants. Such disruption of photosynthetic rate will alter the production of carbohydrate by the host plant. On the face of it, this all appears pretty straightforward. Thus, a plant is attacked, photosynthesis is reduced, and as a result, less carbohydrate is produced. However, as with so much in life, all is not as it appears. Plants might be stationary packages of food, but when they are attacked, there begins, from the very earliest stages, an intricate interaction between host and attacker. Over the past decade, research has revealed just how complex and fascinating these interactions can be. And crucially, carbohydrate metabolism features prominently, as we see in the following sections.

5.2 CARBOHYDRATE PARTITIONING AND METABOLISM IN PLANTS INFECTED BY PATHOGENS

5.2.1 Effects on assimilate partitioning

When examining the effects of pathogens on carbohydrate partitioning and metabolism in the host, it is important to consider the mode of nutrition of the pathogen. As we saw in Chapter 1, biotrophs are organisms that derive their nutrients from the living cells of their hosts. As a result, they must use current assimilates or recently mobilised past assimilates. In contrast, necrotrophs infect living tissues but obtain their nutrients from host tissues killed in advance of colonisation. Of course, classifying pathogens into biotrophs and necrotrophs is an oversimplification, as the nutritional modes of fungi and Oomycetes lie somewhere along the continuum biotrophy–necrotrophy–saprotrophy (Whipps & Lewis, 1981; Newton *et al.*,

2010). Many fungi are hemibiotrophs, because they have an initial biotrophic phase followed by a necrotrophic phase of nutrition.

Despite the fact that biotrophs do not kill host tissues outright, they can still cause considerable damage to the host, resulting in substantial reductions in growth and yield. Such effects appear to be partly the result of the profound effects these pathogens have on the partitioning of newly formed assimilates. In some classic experiments, Livne and Daly (1966) fed $^{14}\text{CO}_2$ to first-formed unifoliate leaves of French beans. In an uninfected, healthy bean plant, 50% of newly fixed carbon was exported from the unifoliate leaf, with 31% going to roots and nearly 6% ending up in young, developing trifoliate leaves. If the unifoliate leaf was infected with the rust, *Uromyces appendiculatus*, export dropped from 50% to just 2%, depriving roots and young leaves of vital assimilates for growth and development. They also fed $^{14}\text{CO}_2$ to healthy trifoliate leaves and found that although import of newly fixed carbon into healthy unifoliate leaves was negligible at just 1%, when the unifoliate leaves were infected with rust, this figure increased to 32%. In this case, young, developing leaves suffered the most. Clearly, rust infection can lead to profound alterations to assimilate partitioning in plants, with serious consequences for growth and yield, as we saw in Chapter 2. The experiments of Livne and Daly (1966) highlighted two important changes in rust infected plants: firstly, assimilate retention in infected, older leaves and secondly, assimilate import into infected leaves. This pioneering work has been confirmed and extended to other biotrophs (see Whipps & Lewis, 1981), but it is worth noting that the effects are less pronounced if the host plant is resistant or if infection intensity is low. Thus, in wheat, when only one leaf of a plant was infected with yellow rust (*Puccinia striiformis*), the translocation pattern of assimilates leaving that leaf was not affected (Doodson *et al.*, 1965). In contrast, when the whole shoot was infected, the translocation pattern was altered, with a greater proportion of assimilate moving to the leaves and considerably less to the roots (Siddiqui & Manners, 1971).

A number of necrotrophic fungi have also been shown to alter translocation patterns in infected hosts by causing assimilate retention at sites of infection, including the wheat glume blotch pathogen, *Septoria nodorum* (Wafford & Whitbread, 1976), and *Alternaria solani*, the cause of leaf blight on tomato (Coffey *et al.*, 1970). Virus infection is also known to alter carbohydrate metabolism and partitioning. Virus-infected source leaves are usually characterised by reduced rates of photosynthesis, decreased concentrations of soluble sugars and starch accumulation (Goodman *et al.*, 1986; Fraser, 1987). However, although reduced photosynthetic rates might be common in virus-infected leaves, sugar levels in such leaves are not always reduced. For example, in melon plants infected with *cucumber mosaic virus* (CMV), photosynthesis was reduced, while levels of glucose and fructose were actually increased significantly above uninfected controls (Shalitin & Wolf, 2000), while in sugar beet infected with the *beet curly top virus* (BCTV), concentrations of sucrose, glucose and starch were increased significantly compared to non-infected controls (Swiech *et al.*, 2001). The mechanisms responsible for these changes in carbohydrate levels in virus-infected sugar beet leaves are discussed in the next section.

5.2.2 Mechanisms associated with altered patterns of partitioning

In the previous section, we have seen that infection by many fungal pathogens can result in assimilate retention in infected leaves and furthermore that this can result in altered partitioning of assimilates. This retention of assimilates in infected leaves was confirmed by researchers

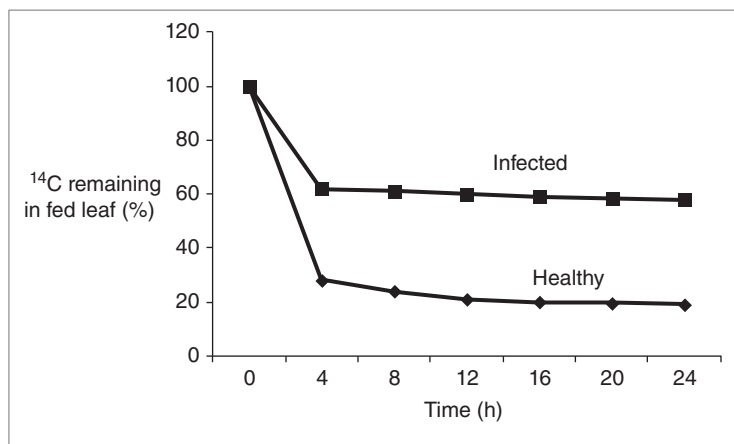


Fig. 5.1 Kinetics of efflux of ^{14}C from healthy and brown rust-infected first leaves of barley following a pulse of labelled CO_2 . Adapted from Oweru *et al.* (1983), with permission of John Wiley and Sons.

feeding a pulse of $^{14}\text{CO}_2$ to leaves of barley infected with brown rust and comparing the amount of carbon remaining in that leaf to that retained by uninfected leaves (Fig. 5.1; Oweru *et al.*, 1983). The efflux of fixed carbon from infected leaves was greatly reduced. Decreased assimilate export from infected leaves can also be inferred from experiments looking at the accumulation of carbon compounds at infection sites after feeding leaves with $^{14}\text{CO}_2$ or feeding detached leaves or leaf disks with ^{14}C -labelled sugars. Indeed, many studies have demonstrated accumulation of carbon compounds at infection sites of biotrophic fungal pathogens, as well as some hemibiotrophs. Studies using labelled CO_2 or sugars have also shown that label originally present in host sucrose, for example, ends up in fungal carbohydrates such as the sugar alcohols (polyols) arabitol, mannitol and erythritol, at pathogen infection sites (Long & Cooke, 1974; Hewitt & Ayres, 1976; Mitchell *et al.*, 1978; see also Whipps & Lewis, 1981). More recently, Voegelé *et al.* (2005) described the expression of a mannitol dehydrogenase (*MADI*) in haustoria of the rust fungus *Uromyces fabae* on broad bean. They found that mannitol concentration increased in apoplastic fluids of infected leaves, as well as in fungal spores. Since polyols can be powerful scavengers of reactive oxygen species (ROS; Shen *et al.*, 1997), it was suggested that the mannitol in the broad bean–rust interaction has two functions: firstly, as a carbohydrate storage compound for the pathogen and secondly, as a scavenger of ROS (Voegelé *et al.*, 2005). Since polyols such as mannitol are not readily used by plant tissues, their synthesis from host carbohydrate maintains a concentration gradient, thereby promoting further carbohydrate uptake from the host (Smith *et al.*, 1969). As Whipps and Lewis (1981) point out, other fungal storage compounds, such as lipids, also accumulate at infection sites of biotrophic fungal pathogens (Lösel & Lewis, 1974; Lösel, 1978), and because they are not freely available to the host, they may also constitute effective sinks for the products of host photosynthesis.

Infection by many powdery mildew and rust fungi results in increased activity of invertase and an accumulation of hexose sugars (and sometimes sucrose) (e.g. Scholes *et al.*, 1994). Increased invertase activity and accumulation of hexoses were also demonstrated in leaves of *Arabidopsis thaliana* infected with the white blister rust pathogen, *Albugo candida* (Fig. 5.2;

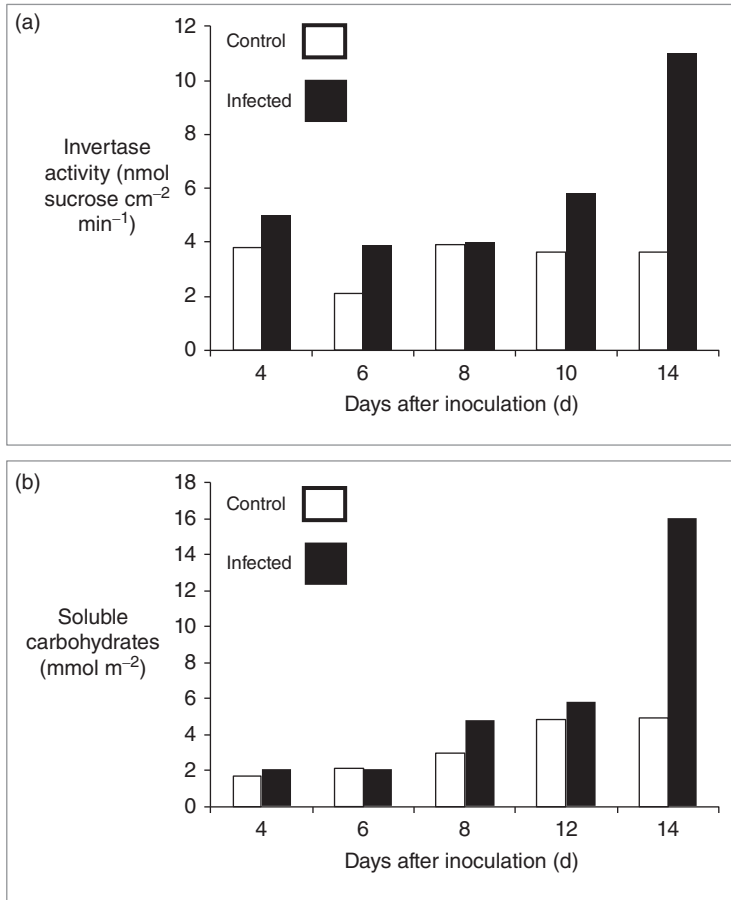


Fig. 5.2 Changes in carbohydrate metabolism in leaves of *Arabidopsis thaliana* inoculated with *Albugo candida*. (a) Alterations in cell-wall-bound invertase activity in leaves of uninfected, control plants and in infected regions of leaves inoculated with *A. candida*. (b) Changes in total soluble carbohydrates (sucrose, glucose and fructose) in leaves of uninfected, control plants and in infected regions of leaves inoculated with *A. candida*. Adapted from Chou *et al.* (2001). Reproduced with permission of John Wiley & Sons.

Chou *et al.*, 2000). In fact, in this interaction, there was an increase in both apoplastic and soluble invertase activities in infected areas of the leaf. The increased activity of apoplastic invertase was shown to be of host origin. Apoplastic invertase is thought to facilitate phloem unloading by maintaining a steep sucrose gradient between photosynthetic cells and sink regions of the plant (Eschrich, 1980), and its activity has been shown to decline as leaves mature, coinciding with the transition from a sink to a source organ (Godt & Roitsch, 1997). Interestingly, in tobacco plants overexpressing invertase in the apoplast, the amount of sucrose exported from the leaf was reduced (Von Schaewen *et al.*, 1990). Thus, increased activity of apoplastic invertase in leaves of *A. thaliana* infected with the white blister rust could reduce sucrose export from the leaf, but it might also facilitate phloem unloading of sucrose into cells

adjacent to pathogen mycelium, thereby changing areas of a source leaf into sinks for carbon. This would aid efficient carbohydrate acquisition by the pathogen (Chou *et al.*, 2000).

In *A. thaliana* infected with *A. candida*, starch content declined in infected regions of the leaf but increased in surrounding areas as infection progressed (Chou *et al.*, 2000). Although a similar pattern was observed in the leaves of *Senecio squalidis* infected with *Albugo trago-pogonis* (Whipps & Cooke, 1978), it contrasts with the pattern of starch accumulation in radish cotyledons infected with *A. candida* and barley leaves infected with brown rust, where accumulated starch was localised to chloroplasts in cells within the pustule (Saettler & Pound, 1966; Scholes & Farrar, 1987). Nevertheless, in leaves of *A. thaliana* infected with *A. candida*, breakdown of starch is likely to have contributed to the increased glucose content in infected regions of the leaf, possibly as a result of an increased demand for carbohydrate by the pathogen during sporulation (Chou *et al.*, 2000).

It is known that sugars control the expression of many plant genes (Koch, 1996). For example, genes encoding photosynthetic proteins and those involved in resource mobilisation are induced by carbohydrate depletion and repressed by the accumulation of sugars or an increase in the flux of sugars through metabolic pathways. As we already know from Chapter 3, leaves infected with obligately biotrophic fungal pathogens, such as powdery mildews and rusts, often exhibit reduced rates of net photosynthesis. Changes in invertase activity, concentrations of hexose sugars and reductions in photosynthesis were linked together in a model, described originally by Scholes (1992) and Scholes *et al.* (1994). In this model, an increase in invertase activity leads to an accumulation of hexose sugars, thereby initiating a signal transduction pathway(s), leading to a repression of photosynthetic gene expression (Fig. 5.3). This, in turn, would result in a reduction in photosynthetic rate. The later work by Chou *et al.* (2000) on *A. thaliana* infected with *A. candida* provided support for this model. This work showed that in infected leaves, increases in invertase activity, the accumulation of hexose sugars and the down-regulation of two photosynthetic genes (*cab* and *rbcS*) were consistent with the sugar sensing model proposed by Sheen (1994) and Jang *et al.* (1997).

In plant–pathogen interactions, down-regulation of photosynthesis, coupled with an increased demand for carbohydrates, often results in a transition of source tissue into sink tissue. Cell wall invertases are important for apoplastic phloem unloading (Roitsch *et al.*, 2003), and so the increased activity of cell wall invertase observed in infected leaves in many plant–pathogen interactions is an indicator of this transition from source to sink status. As pointed out by Berger *et al.* (2007), although down-regulation of photosynthesis and induction of sink metabolism appear to be a general response to pathogen infection, there is variation in effects on sugar levels in different plant–pathogen interactions. Thus, sugar levels have been reported to increase in some interactions (e.g. Wright *et al.*, 1995; Chou *et al.*, 2000; Scharte *et al.*, 2005) but not to change in others (e.g. Berger *et al.*, 2004; Bonfig *et al.*, 2006). It is known that changes in invertase activity and sugar levels are localised in infected regions of leaves in some interactions (e.g. Swarbrick *et al.*, 2006), and measurements made on whole infected leaves will likely miss localised changes in sugar levels (Berger *et al.*, 2007).

So far, we have dealt with changes in invertase activity and sugar levels in compatible interactions. However, cell wall invertase expression and activity and increased sugar levels can also increase during incompatible interactions (Roitsch *et al.*, 2003). Localised increases in hexoses would provide energy for defence reactions but could also trigger expression of defence-related genes, such as *PR-I* (Koch, 1996; Xiao *et al.*, 2000). In tobacco reacting hypersensitively to *Phytophthora nicotianae*, expression of an apoplastic invertase gene was

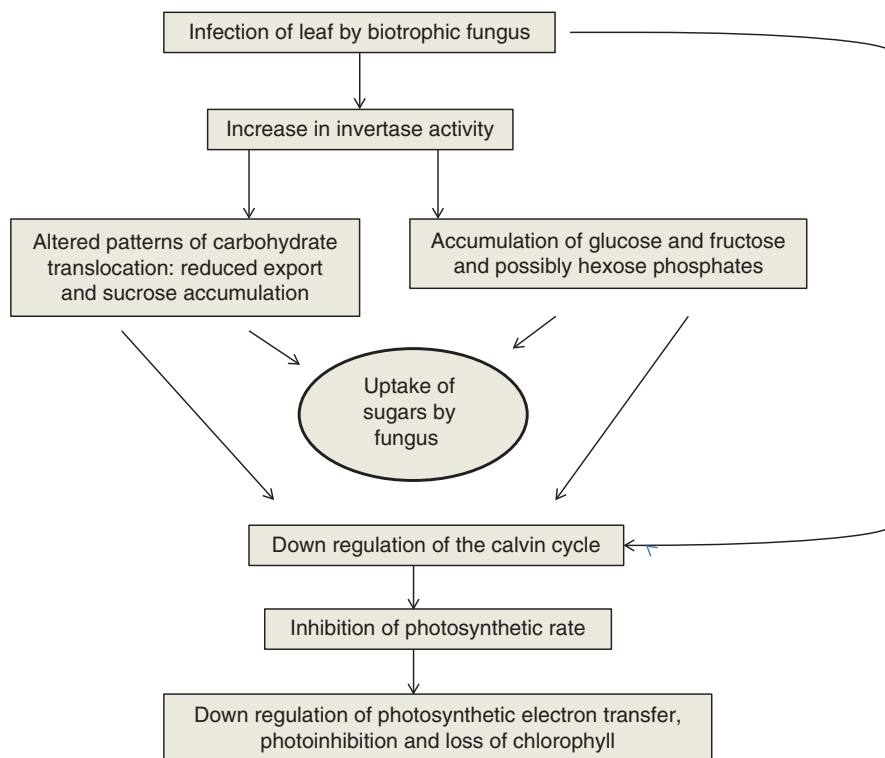


Fig. 5.3 Model of the effects of increased invertase activity and accumulation of carbohydrates on photosynthetic metabolism in leaves infected with a biotrophic fungal pathogen. From Scholes (1992) with permission of the author.

apparent at just 30 minutes after inoculation and was followed by much increased invertase activity some 9 hours after inoculation (Fig. 5.4; Scharte *et al.*, 2005). This was accompanied not only by increased levels of hexoses, but also by increased sucrose levels. It was argued that the increase in sucrose resulted from the defence-induced deposition of callose at the interface of adjacent mesophyll cells within 1 hour of inoculation, which would cause the export of sucrose to collapse and its content in the apoplast to increase (Scharte *et al.*, 2005). The increase in carbohydrate levels in infected cells would have stimulated the metabolic shift of source cells to carbohydrate-consuming or sink activity, but also it induced the expression of a defence-related protein, the sugar-sensitive PR-Q. In a study of both race-specific resistance and broad-spectrum resistance in barley, conditioned by the *Mla12* and *mlo* alleles, respectively, Swarbrick *et al.* (2006) found that apoplastic invertase activity increased in both cases, accompanied by increased levels of hexoses localised to areas of the leaf exhibiting resistance responses. These changes were accompanied by down-regulation of Rubisco (*rbcS*) and up-regulation of PR-1.

The results of the studies described previously are consistent with a role for invertase in generating hexose sugars to fuel defence responses and in acting as signals to induce defence gene expression. What might trigger these increases in invertase activity? In the incompatible

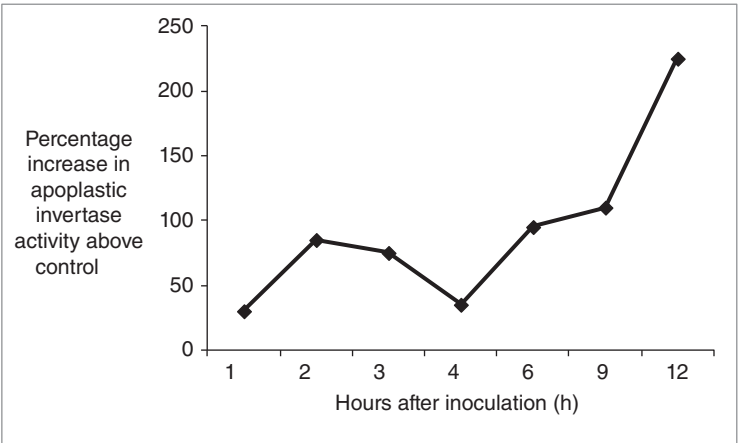


Fig. 5.4 Enhanced activity of apoplastic invertase in leaf tissue inoculated with zoospores of *Phytophthora nicotianae*. Values of apoplastic invertase activity are expressed as percentage increases relative to the uninoculated control. Scharte *et al.* (2005). Reproduced with permission of John Wiley & Sons.

interactions between barley and powdery mildew examined by Swarbrick *et al.* (2006), increases in apoplastic invertase activity were larger and more rapid than those observed in a compatible interaction (Fig. 5.5). This difference in timing suggests that different signals might be responsible for triggering invertase in compatible and incompatible interactions. In the compatible interaction, the increase in invertase was coincident with the increase in fungal biomass, suggesting a metabolic (e.g. sugar) or pathogen-dependent signal (Swarbrick

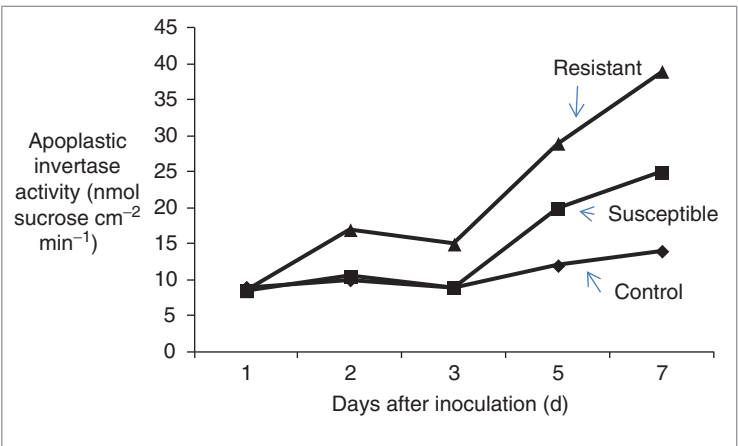


Fig. 5.5 The effect of powdery mildew on apoplastic invertase activity in barley leaves. Invertase activity was determined in leaves of a susceptible variety and a variety expressing broad-spectrum resistance (*mlo*) to powdery mildew. Leaves were inoculated by spraying a suspension of powdery mildew conidia in Fluorinert. Control plants were sprayed with Fluorinert without powdery mildew conidia. Swarbrick *et al.* (2006). Reproduced with permission of John Wiley & Sons.

et al., 2006). In the incompatible interactions, the rapidity of the invertase increase suggests an elicitor-based signal. Interestingly, it was suggested that because cytokinins are known to induce invertase activity and biotrophic fungal pathogens can produce cytokinins, the increases in invertase activity observed in compatible interactions between plants and biotrophic fungal pathogens might be the result of pathogen-derived cytokinins (Walters & McRoberts, 2006).

We saw earlier that concentrations of sucrose, glucose and starch were increased in leaves of sugar beet infected with BCTV compared to uninfected plants (Swiech *et al.*, 2001). These increases occurred in leaves of both susceptible and resistant varieties and were especially large for sucrose (Fig. 5.6), highlighting a profound effect of virus infection on assimilate partitioning in sugar beet. Indeed, the elevated sucrose levels appeared to be the result of disrupted phloem transport out of infected leaves, particularly, an inhibition of sucrose loading into the phloem of minor veins. These changes were consistent with physical changes to vascular tissue caused by BCTV infection (Esau & Hoefert, 1978). The increase in glucose in virus-infected leaves appeared to be the result of increased acid invertase activity (Swiech *et al.*, 2001), which would break down sucrose to glucose and fructose, neither of which is readily loaded into the phloem (Sonnewald *et al.*, 1994).

An increase in soluble sugars (sucrose, glucose and fructose) was also found in tobacco leaves infected with potato virus Y (PVY; Herbers *et al.*, 2000). In this case, the increased sucrose appeared to be due to the increased activity of cell wall invertase, because, as we saw in the previous paragraph, sucrose breakdown to glucose and fructose would prohibit its loading into the phloem. Interestingly, the increases in soluble sugars were accompanied by induction of defence responses, which could have been responsible for the increased resistance to PVY attack observed in previous work (Herbers *et al.*, 1996).

Most viruses encode a protein that is essential for cell-to-cell spread of virus particles in infected plants. They are known as movement proteins (MP) and are known to localise to secondary branched plasmodesmata, to greatly increase the plasmodesmatal size exclusion limit and to facilitate cell-to-cell transfer of MP-bound virus genomes through plasmodesmata (Lucas, 2006). MPs can also affect sugar metabolism and transport in plants, although the

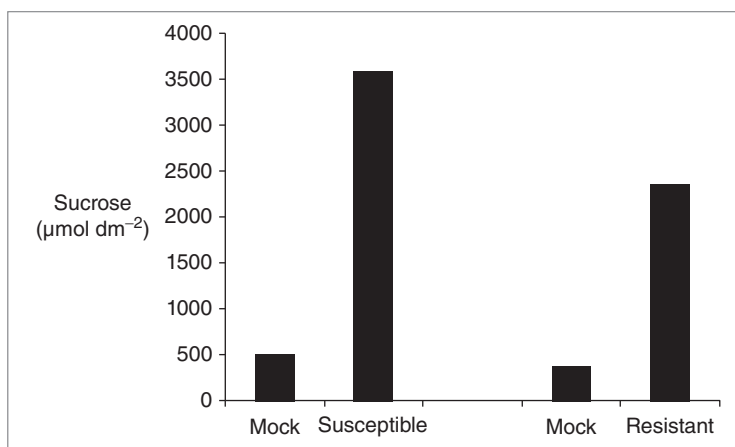


Fig. 5.6 Sucrose accumulation in leaves of the sugar beet varieties Z-10 (susceptible) and 9BB6090 (resistant) inoculated with sugar BCTV. Swiech *et al.* (2001). Reproduced with permission from Elsevier.

final effect can vary depending on the plant species, tissue specificity of the promoter used and plant developmental stage. For example, ectopic expression of TMV-MP under the control of a constitutive promoter in transgenic tobacco led to carbohydrate accumulation and reduced export of sucrose from source leaves (e.g. Balachandran *et al.*, 1995), while expression of TMV-MP from the green tissue-specific ST-LS1 promoter in potato led to lower carbohydrate content and increased sucrose export (Olesinski *et al.*, 1996). In a later study, Kronberg *et al.* (2007) examined the effects of expressing the potato leaf roll virus MP (MP17), fused to green fluorescent protein, in different ecotypes of *Arabidopsis*. They found that low level accumulation of MP17 led to enhanced efflux of sucrose from source leaves and greatly increased production of vegetative biomass, whereas high levels of MP17 expression disrupted sucrose export, leading to carbohydrate accumulation and reduced vegetative growth. Rather surprisingly, this inhibition of carbohydrate partitioning was subsequently reversed as plants developed, leading to a 30% increase in seed yield in transgenic plants compared to wild types (Kronberg *et al.*, 2007). This reversal of the assimilate export block was accompanied by reduced accumulation of MP17 in plasmodesmata, suggesting major structural changes in plasmodesmata during leaf senescence.

5.3 CARBOHYDRATE METABOLISM AND PARTITIONING IN PLANT-INSECT HERBIVORE INTERACTIONS

It is well known that herbivory causes large-scale changes in the expression of genes related to primary and secondary metabolism (e.g. Hermsmeier *et al.*, 2001; Mercke *et al.*, 2004; Reymond *et al.*, 2004). Some of these changes are related to synthesis of defensive compounds locally and/or systemically and changes in resource capture and partitioning. Alterations in carbon assimilation and partitioning are important as plants prepare for defence, as carbon skeletons and energy are required for biosynthetic reactions. Indeed, various studies have suggested that rapidly shifting patterns of assimilate partitioning are an important part of plant defensive responses. Jasmonates are known to mimic some aspects of plant defence (e.g. Baldwin, 1990; Howe, 2005) and have been used to study defence responses. For example, treatment of hybrid poplar with jasmonic acid increased partitioning of assimilates in mature leaves to young sink leaves (Arnold & Schultz, 2002). The authors suggested that this altered partitioning provided resources for increased formation of tannins. Later work found that when leaves of *Populus nigra* were treated with jasmonic acid, photosynthate export from both treated and untreated leaves increased, with increased partitioning to stems and roots, suggesting that plants partition recently fixed carbon to tissues that are inaccessible to herbivores attacking leaves (Babst *et al.*, 2005). Increased partitioning of photosynthate to stems and roots was also found in *Nicotiana attenuata* after treatment of leaves with regurgitant of the specialist herbivore, *Manduca sexta* (Schwachtje *et al.*, 2006). This response was shown to be regulated by GAL83, which is the β -subunit of a SnRK1 (SNF1-related kinase) protein kinase. Interestingly, SnRK1s function as cellular fuel gauges, regulating several key enzymes in sugar metabolism (e.g. Halford *et al.*, 2004). It appears that when *N. attenuata* is attacked by *M. sexta*, there is an increased partitioning of newly fixed carbon to roots, enhancing root reserves and rendering it inaccessible to the folivore. This enables the plant to better tolerate attack by *M. sexta*, by providing increased root reserves that can be used to sustain seed production at the end of the plant's life, after the larvae of the herbivore have pupated (Schwachtje *et al.*, 2006).

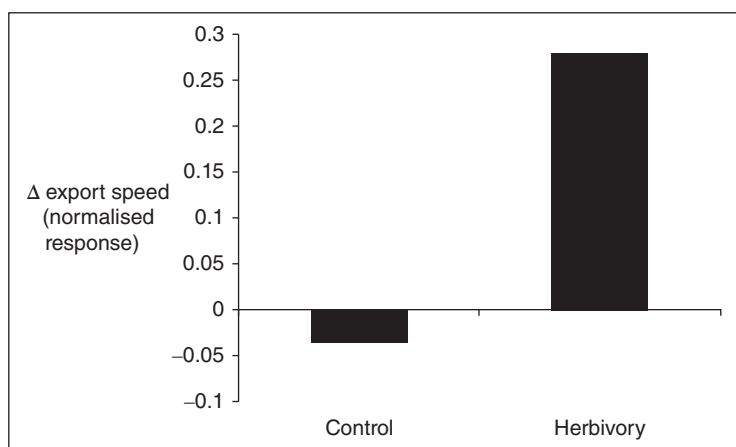


Fig. 5.7 Normalised response of ^{11}C export speed from systemic poplar leaves 18 hours after herbivory by caterpillars of the gypsy moth, *Lymantria dispar*. Export speed was measured as the distance between the leaf exposed to $^{11}\text{CO}_2$ and the detector placed on the stem, divided by the time between $^{11}\text{CO}_2$ administration to the leaf and the arrival of ^{11}C at the first radiation detector on the stem. Normalised response is the change in export speed after treatment as a proportion of the pre-treatment export speed. Babst *et al.* (2008). Reproduced with permission of John Wiley & Sons.

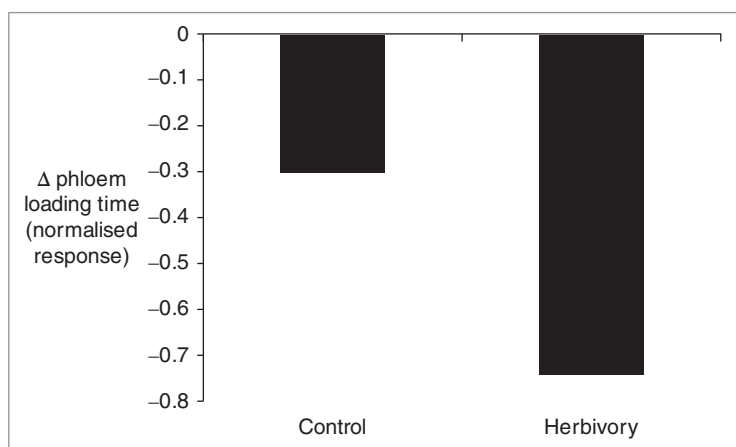


Fig. 5.8 Normalised response of phloem loading time (transit time of ^{11}C through the poplar leaf, starting at exposure to $^{11}\text{CO}_2$ and including all processes through phloem loading). Loading time was calculated on the basis of the export speed and the transport speed through the phloem in the stem. Transport speed was calculated as the distance between two detectors divided by the time taken for ^{11}C -photosynthate to travel from the first to the second detector. Normalised response was the change after treatment as a proportion of the pre-treatment measurement. Babst *et al.* (2008). Reproduced with permission of John Wiley & Sons.

A later study by Babst *et al.* (2008) provided evidence for rapid changes in export and partitioning of carbon in *P. nigra* subjected to herbivory by the gypsy moth, *Lymantria dispar*. In this case, carbon partitioning from young, undamaged leaves increased substantially just 18 hours after low level herbivore damage on older leaves. This was accompanied by an increase in the speed of export of carbon from undamaged leaves (Fig. 5.7), as a result of a reduction in phloem loading time (Fig. 5.8). The authors suggested that because the major defensive chemicals in *P. nigra* are found in leaves, the increased partitioning of carbon to stems and roots might enable plants to tolerate leaf damage, by providing enhanced reserves that could be accessed once the herbivore attack subsided (Babst *et al.*, 2008).

Some workers (Steinbrenner *et al.*, 2011; Gómez *et al.*, 2012) have suggested that the lack of evidence for actual accumulation of carbohydrate resources in roots argues against the proposal that herbivory-induced resource sequestration is a tolerance mechanism, working by increasing root reserves for future regrowth (Schwachtje *et al.*, 2006).

5.4 CARBOHYDRATE METABOLISM AND PARTITIONING IN INTERACTIONS BETWEEN PLANTS AND PARASITIC ANGIOSPERMS

Many parasitic plants, particularly holoparasites, compete directly with the host for assimilates. Good examples are holoparasites from the genus *Orobanchae*, which parasitize a wide

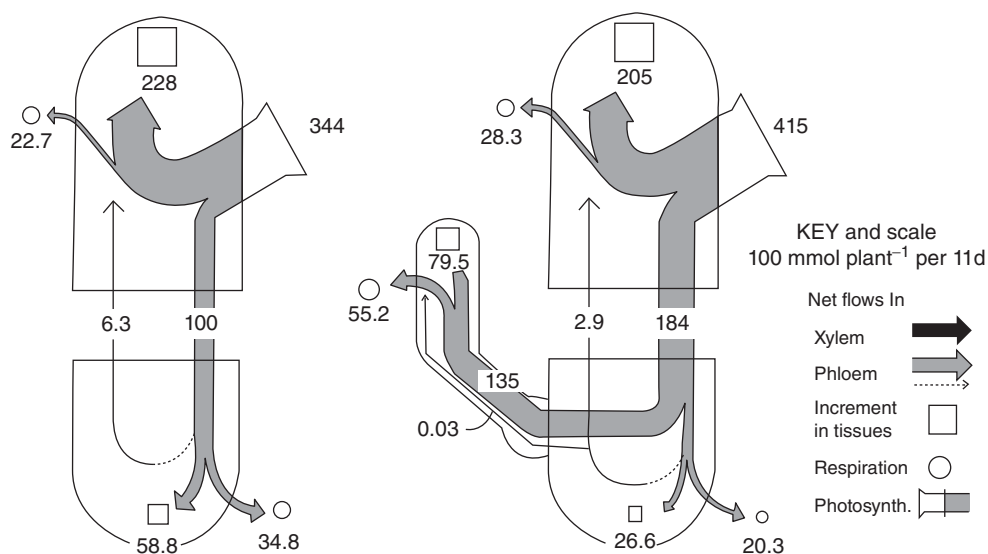


Fig. 5.9 Empirical model of the net carbon flows in the xylem (black arrows) and in the phloem (dotted arrows) over an 11-day study period in uninfected tobacco and tobacco infected by the root parasitic angiosperm *Orobancha cernua*. The width of arrows (net flows via xylem and phloem), area of squares (carbon increments) and area of circles (carbon lost through respiration) are drawn in proportion to the rates of flows, increments or losses. Hibberd *et al.* (1999). Reproduced with permission of John Wiley & Sons.

range of hosts. In a detailed study of solute fluxes in tobacco infected with *O. cernua*, Hibberd *et al.* (1999) found that infected plants fixed 20% more carbon than uninfected controls, with a much larger proportion of the fixed carbon diverted to host roots (84% increase). Incredibly, despite the increased movement of fixed carbon to roots of infected plants, these roots received less carbon than those of control plants, because the parasite removed 73% of the carbon supplied to the roots (Fig. 5.9). Moreover, some 99% of the carbon removed by *O. cernua* is likely to have been delivered via the phloem. *O. cernua* influenced host carbon fluxes in a similar manner to the shoot parasite *Cuscuta reflexa* (Jeschke *et al.*, 1994), and indeed, both parasites have a high dependence on the host phloem for supplies of solutes. In contrast, the hemiparasite, *Oxalis phyllanthi*, was more dependent on the host xylem, which supplied 44% of the host carbon removed from its *Acacia* host (Tennakoon *et al.*, 1997). This highlights a point already made in Chapter 4 that despite being chlorophyllous, hemiparasites such as *O. phyllanthi* and *Striga* species can still rely heavily on the host for its carbon requirements. Indeed, it has been estimated that both *O. phyllanthi* and *Striga* access approximately 35% of their respective host's non-structural carbon (Press *et al.*, 1987; Tennakoon & Pate 1996), while abstraction of carbon by *O. phyllanthi* was quantified at 27% of recently fixed photosynthate (Tennakoon *et al.*, 1997). However, the proportion of host assimilate used by the parasite has been shown to vary with respect to nitrogen status of the host, with *Striga* on sorghum obtaining a greater proportion of host-derived carbon under nitrogen-limiting conditions (Cechin & Press, 1993).

5.5 CONCLUSIONS

It used to be thought that in the interaction between a plant and an attacker, changes in plant primary metabolism (e.g. photosynthesis and carbohydrate metabolism) were mere consequences of the interaction; any effective responses by the plant, usually viewed in terms of resistance, were thought to revolve around secondary metabolism, involving the synthesis of defensive compounds, for example (Schwachtje & Baldwin, 2008). We now know that this is just not true. Thanks to the development of transcriptomic, metabolomic and proteomic approaches to studying plant responses to their environment, it is increasingly clear that changes in primary metabolism are central to interactions between a plant and its attacker. Various studies within the last decade have demonstrated changes in the expression of hundreds of genes, involved in both primary and secondary metabolism, suggesting a major reconfiguration of host metabolism under attack. If plants are capable of mounting an effective defence, the formation of defences will require resources, and this in turn could lead to a reduction in host growth and reproduction (Fig. 5.10; Schwachtje & Baldwin, 2008). There might even be increases in carbon assimilation in an attempt to meet the increased demand for energy and carbon skeletons. However, changes in host primary metabolism might also help plants to tolerate ravages of pathogen or herbivore attack (Fig. 5.10). Moreover, some primary metabolites, for example sucrose, glucose and fructose, have been shown to function in defence signalling (Ahn *et al.*, 1996; Ahn & Lee, 2003).

Plants are amazing survivors, capable of using their incredible biosynthetic potential to deal with the biotic and abiotic environment. Determining how plants use and coordinate this metabolic potential under attack will provide exciting times for researchers in the future.

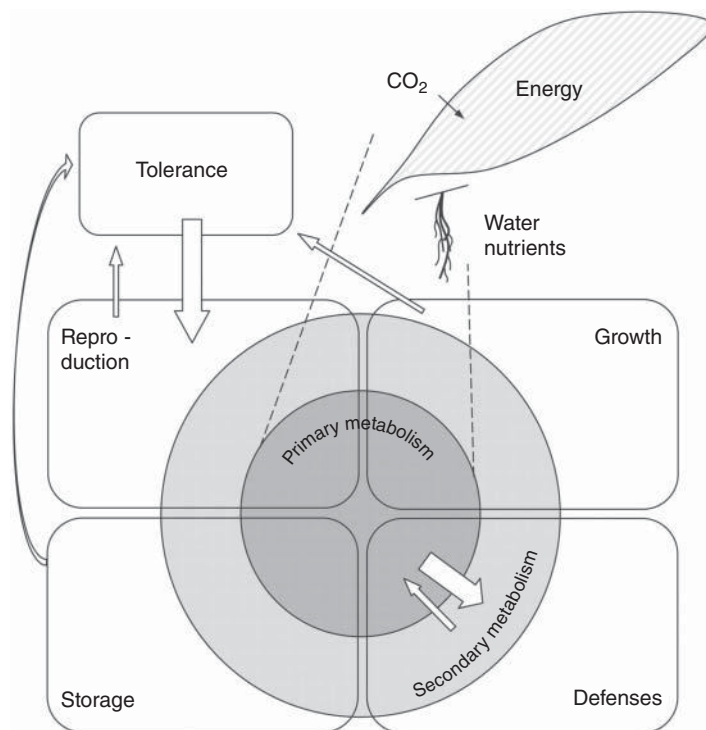


Fig. 5.10 Dependency of resistance traits (defences and tolerance) and primary metabolism. Primary metabolism is fuelled by energy and resources, which the plant gains from its environment. Primary metabolism involves growth, storage and reproduction. Tolerance depends on primary metabolites and energy, both of which are taken from pools for reproduction, storage and/or growth and later reinvested in reproduction. Defences from secondary metabolism are based on energy and resources from primary metabolism, which can be partially resupplied to primary metabolism. Parts of primary metabolism can function as direct defence. Schwachtje and Baldwin (2008). Reproduced with permission of American Society of Plant Biologists.

RECOMMENDED READING

- Chou H-M, Bundock N, Rolfe SA, Scholes JD, 2000. Infection of *Arabidopsis thaliana* with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. *Molecular Plant Pathology* **1**, 99–113.
- Livne A, Daly JM, 1966. Translocation in healthy and rust-affected beans. *Phytopathology* **56**, 170–175.
- Scholes JD, Lee PJ, Horton P, Lewis DH, 1994. Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. *New Phytologist* **126**, 213–222.
- Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT, 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12935–12940.
- Schwachtje J, Baldwin IT, 2008. Why does herbivore attack reconfigure primary metabolism? *Plant Physiology* **146**, 845–851.

REFERENCES

- Ahn JH, Choi Y, Kwon YM, Kim SG, Choi YD, Lee JS, 1996. A novel extension gene encoding a hydroxyproline-rich glycoprotein requires sucrose for its wound-inducible expression in transgenic plants. *Plant Cell* **8**, 1477–1490.
- Ahn JH, Lee JS, 2003. Sugar acts as a regulatory signal on the wound-inducible expression of SbHRGP3::GUS in transgenic plants. *Plant Cell Reporter* **22**, 286–293.
- Arnold TM, Schultz JC, 2002. Induced sink strength as a prerequisite for induced tannin biosynthesis in developing leaves of *Populus*. *Oecologia* **130**, 585–593.
- Babst BA, Ferreiri RA, Gray DW, Lerdau M, Schyler DJ, Schueller M, Thorpe MR, Orians CM, 2005. Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. *New Phytologist* **167**, 63–72.
- Babst BA, Ferreiri RA, Thorpe MR, Orians CM, 2008. *Lymantria dispar* herbivory induces rapid changes in carbon transport and partitioning in *Populus nigra*. *Entomologia Experimentalis et Applicata* **128**, 117–125.
- Balachandran S, Hull RJ, Vaadia Y, Wolf S, Lucas WJ, 1995. Alteration in carbon partitioning induced by the movement protein of tobacco mosaic virus originates in the mesophyll and is independent of change in the plasmodesmatal size exclusion limit. *Plant, Cell and Environment* **18**, 1301–1310.
- Baldwin IT, 1990. Herbivory simulations in ecological research. *Trends in Ecology and Evolution* **5**, 91–93.
- Berger S, Papadopoulos M, Schreiber U, Kaiser W, Roitsch T, 2004. Complex regulation of gene expression, photosynthesis and sugar levels by pathogen infection in tomato. *Physiologia Plantarum* **122**, 419–428.
- Berger S, Benediktyová Z, Matouš K, Bonfig K, Mueller MJ, Nedbal L, Roitsch T, 2007. Visualization of dynamics of plant-pathogen interaction by novel combination of chlorophyll fluorescence imaging and statistical analysis: differential effects of virulent and avirulent strains of *P. syringae* and of oxylipins on *A. thaliana*. *Journal of Experimental Botany* **58**, 797–806.
- Bonfig K, Schreiber U, Gabler A, Roitsch T, Berger S, 2006. Infection with virulent and avirulent *P. syringae* strains differentially affects photosynthesis and sink metabolism in *Arabidopsis* leaves. *Planta* **225**, 1–12.
- Cechin I, Press MC, 1993. Influence of nitrogen on growth and photosynthesis of a C3 cereal, *Oryza sativa*, infected with the root hemiparasite *Striga hermonthica*. *Journal of Experimental Botany* **45**, 925–930.
- Chou H-M, Bundock N, Rolfe SA, Scholes JD, 2000. Infection of *Arabidopsis thaliana* with *Albugo candida* (white blister rust) causes a reprogramming of host metabolism. *Molecular Plant Pathology* **1**, 99–113.
- Coffey MD, Marshall C, Whitbread R, 1970. The translocation of ¹⁴C-labelled assimilates in tomato plants infected with *Alternaria Solani* (Ell. and Mart.) Jones and Grout. *Annals of Botany* **34**, 605–615.
- Doodson JK, Manners JG, Myers A, 1965. Some effects of yellow rust (*Puccinia striiformis*) on ¹⁴carbon assimilation and translocation in wheat. *Journal of Experimental Botany* **16**, 304–317.
- Esau K, Hoefert LL, 1978. Hyperplastic phloem in sugar beet leaves infected with the beet curly top virus. *American Journal of Botany* **65**, 772–783.
- Eschrich W, 1980. Free space invertase, its possible role in phloem unloading. *Berichte der Deutschen Botanischen Gesellschaft* **93**, 363–378.
- Fraser RSS, 1987. *Biochemistry of virus-infected plants*. Letchworth, Hertfordshire, UK: Research Studies Press.
- Godt DE, Roitsch T, 1997. Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggests an important function in establishing and maintaining sink metabolism. *Plant Physiology* **115**, 273–282.
- Goodman RN, Király Z, Wood KR, 1986. *The biochemistry and physiology of plant disease*. Columbia, Missouri: University of Missouri Press.
- Gómez S, Steinbrenner AD, Osorio S, Schueller M, Ferreiri RA, Fernie AR, Orians CM, 2012. From shoots to roots: transport and metabolic changes in tomato after simulated feeding by a specialist lepidopteran. *Entomologia Experimentalis et Applicata* **144**, 101–111.
- Halford NG, Hey S, Jhurreea D, Laurie S, McKibbin RS, Zhang Y, Matthew JP, 2004. Highly conserved protein kinases involved in the regulation of carbon and amino acid metabolism. *Journal of Experimental Botany* **55**, 35–42.
- Herbers K, Meuwly P, Frommer WBM, Métraux J-P, Sonnewald U, 1996. Systemic acquired resistance mediated by the ectopic expression of invertase: possible hexose sensing in the secretory pathway. *Plant Cell* **8**, 793–803.

- Herbers K, Takahata Y, Melzer M, Mock H-P, Hajirezaei M, Sonnewald U, 2000. Regulation of carbohydrate partitioning during the interaction of potato virus Y with tobacco. *Molecular Plant Pathology* **1**, 51–59.
- Hermesmeier D, Schittko U, Baldwin IT, 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology* **125**, 683–700.
- Hewitt HG, Ayres PG, 1976. Effects of infection by *Microsphaera alphitoides* (powdery mildew) on carbohydrate levels and translocation in seedlings of *Quercus robur*. *New Phytologist* **77**, 379–390.
- Hibberd JM, Quick WP, Press MC, Scholes JD, Jeschke WD, 1999. Solute fluxes from tobacco to the parasitic angiosperm *Orobancha cernua* and the influence of infection on host carbon and nitrogen relations. *Plant, Cell and Environment* **22**, 937–947.
- Howe GA, 2005. Jasmonates as signals in the wound response. *Journal of Plant Growth Regulation* **23**, 233–237.
- Jang J, León P, Zhou L, Sheen J, 1997. Hexokinase as a sugar sensor in higher plants. *Plant Cell* **9**, 5–19.
- Jeschke WD, Bäuml P, R  th N, Czygan F-C, Proksch P, 1994. Modelling the flow and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. *Journal of Experimental Botany* **45**, 801–812.
- Koch KE, 1996. Carbohydrate-modulated gene expression in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 509–540.
- Kronberg K, Vogel F, Ruten T, Hajirezaei M-R, Sonnewald U, Hofius D, 2007. The silver lining of a viral agent: increasing seed yield and harvest index in Arabidopsis by ectopic expression of the potato leaf roll virus movement protein. *Plant Physiology* **145**, 905–918.
- Livne A, Daly JM, 1966. Translocation in healthy and rust-affected beans. *Phytopathology* **56**, 170–175.
- Long DE, Cooke RC, 1974. Carbohydrate composition and metabolism of *Senecio squalidus* L. leaves infected with *Albugo tragopogonis* (Pers.) S.F. Gray. *New Phytologist* **73**, 889–899.
- L  sel DM, 1978. Lipid metabolism of leaves of *Poa pratensis* during infection by *Puccinia poarum*. *New Phytologist* **80**, 167–174.
- L  sel DM, Lewis DH, 1974. Lipid metabolism in leaves of *Tussilago farfara* during infection by *Puccinia poarum*. *New Phytologist* **73**, 1157–1169.
- Lucas WJ, 2006. Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virology* **344**, 169–184.
- Mercke P, Kappers IF, Verstappen FWA, Vorst O, Dicke M, Boumeester HJ, 2004. Combined transcript and metabolite analysis reveals genes involved in spider mite induced volatile formation in cucumber plants. *Plant Physiology* **135**, 2012–2024.
- Mitchell DT, Fung AK, Lewis DH, 1978. Changes in the ethanol-soluble carbohydrate composition and acid invertase in infected first leaf tissues susceptible to crown rust of oat and wheat stem rust. *New Phytologist* **80**, 381–392.
- Newton AC, Fitt BDL, Atkins SD, Walters DR, Daniells TJ, 2010. Pathogenesis, parasitism and mutualism in the trophic space of microbe-plant interactions. *Trends in Microbiology* **18**, 365–373.
- Olesinski AA, Almon E, Navot N, Perl A, Galun E, Lucas WJ, Wolf S, 1996. Tissue-specific expression of the tobacco mosaic virus movement protein in transgenic potato plants alters plasmodesmatal function and carbohydrate partitioning. *Plant Physiology* **111**, 541–550.
- Owera SAP, Farrar JF, Whitbread R, 1983. Translocation from leaves of barley infected with brown rust. *New Phytologist* **94**, 111–123.
- Press MC, Shah N, Tuohy JM, Stewart GR, 1987. Carbon isotope ratios demonstrate carbon flux from C4 host to C3 parasite. *Plant Physiology* **85**, 1143–1145.
- Reymond P, Bodenhausen N, van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE, 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *The Plant Cell* **16**, 3132–3147.
- Roitsch T, Balibrea ME, Hofmann M, Proels R, Sinha AK, 2003. Extracellular invertase: key metabolic enzyme and PR protein. *Journal of Experimental Botany* **54**, 513–524.
- Saettler AW, Pound GS, 1966. Photosynthesis, carbohydrate levels, and translocation in radish leaves infected by *Albugo candida*. *Phytopathology* **56**, 898.
- Scharte J, Sch  n H, Weis E, 2005. Photosynthesis and carbohydrate metabolism in tobacco leaves during an incompatible interaction with *Phytophthora nicotianae*. *Plant, Cell and Environment* **28**, 1421–1435.
- Scholes JD, 1992. Photosynthesis: cellular and tissue aspects in diseased leaves. In: Ayres PG, ed. *Pests and pathogens*. Oxford, UK: BIOS Scientific Publishers Limited, pp. 85–105.

- Scholes JD, Farrar JF, 1987. Development of symptoms of brown rust of barley in relation to the distribution of fungal mycelium, starch accumulation and localized changes in the concentration of chlorophyll. *New Phytologist* **107**, 103–117.
- Scholes JD, Lee PJ, Horton P, Lewis DH, 1994. Invertase: understanding changes in the photosynthetic and carbohydrate metabolism of barley leaves infected with powdery mildew. *New Phytologist* **126**, 213–222.
- Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT, 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 12935–12940.
- Schwachtje J, Baldwin IT, 2008. Why does herbivore attack reconfigure primary metabolism? *Plant Physiology* **146**, 845–851.
- Shalitin D, Wolf S, 2000. Cucumber mosaic virus infection affects sugar transport in melon plants. *Plant Physiology* **123**, 597–604.
- Sheen J, 1994. Feedback control of gene expression. *Photosynthesis Research* **39**, 427–438.
- Shen B, Jensen RG, Bohnert HJ, 1997. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis in chloroplasts. *Plant Physiology* **113**, 1177–1183.
- Siddiqui MQ, Manners JG, 1971. Some effects of general yellow rust (*Puccinia striiformis*) infection on ^{14}C carbon assimilation and growth in spring wheat. *Journal of Experimental Botany* **22**, 792–799.
- Smith D, Muscatine L, Lewis D, 1969. Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biological Reviews* **44**, 17–90.
- Sonnewald U, Lerchl J, Zrenner R, Frommer W, 1994. Manipulation of sink-source relations in transgenic plants. *Plant, Cell and Environment* **17**, 649–658.
- Steinbrenner AD, Gomez S, Osorio S, Fernie AR, Orians CM, 2011. Herbivore-induced changes in tomato (*Solanum lycopersicum*) primary metabolism: a whole plant perspective. *Journal of Chemical Ecology* **37**, 1294–1303.
- Swarbrick PJ, Schulze-Lefert P, Scholes JD, 2006. Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. *Plant, Cell and Environment* **29**, 1061–1076.
- Swiech R, Browning S, Molsen D, Stenger DC, Holbrook GP, 2001. Photosynthetic responses of sugar beet and *Nicotiana benthamiana* Domin. infected with beet curly top virus. *Physiological and Molecular Plant Pathology* **58**, 43–52.
- Tennakoon KU, Pate JS, 1996. Heterotrophic gain of carbon from hosts by the xylem-tapping root hemiparasite *Oxalophyllanthi* (Oxalaceae). *Oecologia* **105**, 369–376.
- Tennakoon KU, Pate JS, Fineran BA, 1997. Growth and partitioning of carbon and fixed nitrogen in the shrub legume *Acacia littorea* in the presence or absence of the root hemiparasite *Oxalophyllanthi*. *Journal of Experimental Botany* **48**, 1047–1060.
- Voegele RT, Hahn M, Lohaus G, Link T, Heiser I, Mendgen K, 2005. Possible roles for mannitol and mannitol dehydrogenase in the biotrophic plant pathogen *Uromyces fabae*. *Plant Physiology* **137**, 190–198.
- Von Schaewen A, Stitt M, Schmidt R, Sonnewald U, Willmitzer L, 1990. Expression of a yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. *EMBO Journal* **9**, 3033–3044.
- Wafford JD, Whitbread R, 1976. Effects of leaf infections by *Septoria nodorum* Berk. On the translocation of ^{14}C -labelled assimilates in spring wheat. *Annals of Botany* **40**, 83–90.
- Walters DR, McRoberts N, 2006. Plants and biotrophs: a pivotal role for cytokinins? *Trends in Plant Science* **11**, 581–586.
- Whipps JM, Cooke RC, 1978. Comparative physiology of *Albugo tragopogonis*-infected and *Puccinia lagenophorae*-infected plants of *Senecio squalidus* L. *New Phytologist* **81**, 307–319.
- Whipps JM, Lewis DH, 1981. Patterns of translocation, storage and interconversion of carbohydrates. In: Ayres PG, ed. *Effects of disease on the physiology of the growing plant*. Cambridge: Cambridge University Press, pp. 47–83.
- Wright DP, Baldwin BC, Shephard MC, Scholes JD, 1995. Source-sink relationships in wheat leaves infected with powdery mildew. I. Alterations in carbohydrate metabolism. *Physiological and Molecular Plant Pathology* **47**, 237–253.
- Xiao WY, Sheen J, Jang JC, 2000. The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Molecular Biology* **44**, 451–461.

6 Water Relations of Plants Attacked by Pathogens, Insect Herbivores and Parasitic Plants

6.1 INTRODUCTION

Plant water relations can be greatly affected by pathogen infection, insect attack and infection by parasitic angiosperms. For example, wilting is a common symptom of pathogen infection, although the physiological basis of the wilting will depend on the particular host–pathogen interaction. Thus, foliar infection might increase transpiration rate, while root infection might destroy root tissue, and infection by vascular wilt pathogens will block xylem vessels, thereby reducing water flow from root to shoot. Disruption of plant water status can affect uptake and transport of nutrients (see Chapter 7) and can also lead to water stress, with consequences for plant metabolism, growth and yield. Infection- or attack-induced water stress can put affected plants at a competitive disadvantage compared to un-infected or un-infested neighbours and can also exacerbate the effects of abiotic stresses, such as low temperatures.

When writing this chapter, I have assumed that the reader will be familiar with the basic principles of plant cell water relations, transpiration and water uptake by plant roots. For those who need to reacquaint themselves with these topics, excellent accounts are provided by Öpik and Rolfe (2005), Scott (2008) and Smith *et al.* (2010).

6.2 EFFECTS OF PATHOGENS ON PLANT WATER RELATIONS

6.2.1 Foliar pathogens

Leaves lose water via transpiration because of the large difference in water potential between leaf cells and the surrounding atmosphere. This gradient in water potential provides the main driving force for water movement in plants. The hydrophobic cuticle covering leaves provides a highly effective barrier against water loss, and indeed, most water is lost from leaves via the stomata, which must open to allow CO₂ entry for photosynthesis. Should water be in short supply, stomatal closure can be induced rapidly by highly efficient signalling between roots and stomatal guard cells (Wilkinson & Davies, 2002). Pathogens that infect leaves can disrupt

this cuticular and stomatal control of water loss in various ways (Grimmer *et al.*, 2012), which will be considered in the following sections.

6.2.1.1 Alterations in stomatal aperture

Altered stomatal behaviour is a common feature of foliar infections, with reduced stomatal aperture in the light reported in a range of host–pathogen interactions (Grimmer *et al.*, 2012). For example, reduced stomatal opening was found in leaves of French bean inoculated with the biotrophic rust fungus *Uromyces appendiculatus* and the hemibiotrophic anthracnose pathogen *Colletotrichum lindemuthianum* (Lopes & Berger, 2001). In this case, reductions in stomatal opening in bean infected with *C. lindemuthianum* were related to disease severity, with very substantial reductions in stomatal conductance associated with low levels of anthracnose severity (Lopes & Berger, 2001). Infection of barley with the biotrophic powdery mildew fungus, *Blumeria graminis* f.sp. *hordei*, has also been reported to lead to reduced stomatal opening in the light (Ayres & Zadoks, 1979; Prats *et al.*, 2006). In the latter work, inoculation of the susceptible barley cultivar Pallas with powdery mildew led to significant reductions in stomatal conductance in the light but not in the dark (Fig. 6.1; Prats *et al.*, 2006). These effects on stomatal opening were observed within 2–4 hours after inoculation, well before maturation of the appressoria or infection structures of the powdery mildew. Exactly how these changes in stomatal opening could be induced this early in the host–pathogen interaction is not known, although host cell responses can be detected within 2 hours of contact of the primary germ tube of the powdery mildew fungus with the barley leaf (Zeyen *et al.*, 2002). More recently, Koers *et al.* (2011) found that infection of barley by the powdery mildew fungus triggers stomatal closure by stimulating S-type (slow) anion channels in guard cells. As a result, guard cells extrude K^+ salts, leading to stomatal closure.

Pathogen infection can also lead to increased stomatal opening. In some early work on stomatal behaviour in plant–pathogen interactions, Farrell *et al.* (1969) found that infection of

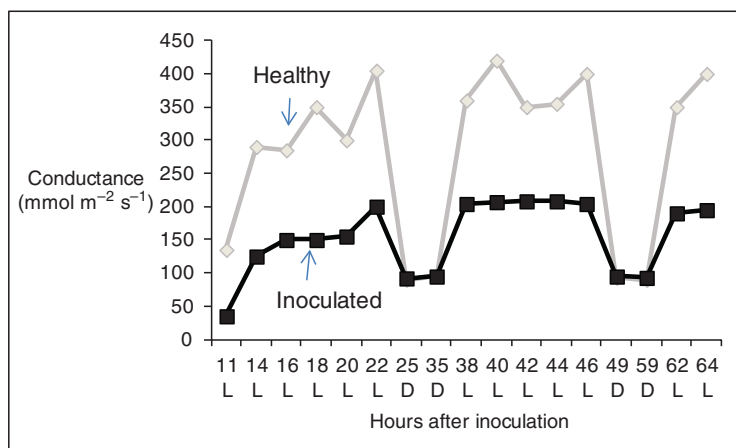


Fig. 6.1 Time-course of stomatal conductance of leaves of the susceptible barley genotype Pallas following inoculation with powdery mildew. Measurements were made during the light (L = light) and the dark (D = dark). Adapted from Prats *et al.* (2006). Reproduced with permission of Oxford University Press.

potato by the late blight pathogen *Phytophthora infestans* induced stomata to open more widely than normal in the light and to remain open in the dark, leading to increased transpiration. Inoculation of barley leaves with the hemibiotrophic fungus *Rhynchosporium secalis* (now *R. commune*) was found to increase stomatal opening at infection sites (Ayres, 1972). Initially, this fungus grows subcuticularly and causes an increase in the permeability of underlying leaf cells (Jones & Ayres, 1972). This causes epidermal cells to lose solutes, thereby altering turgor relations between stomatal guard cells and surrounding epidermal cells, leading to increased stomatal opening in the light. In subsequent work, Ayres and Jones (1975) found that stomata at infection sites also failed to close in the dark (Fig. 6.2a). The result of this increased stomatal opening in both light and dark was a very much increased transpiration rate (Fig. 6.2b). Inhibition of stomatal closure in the dark also occurs in leaves of *Vicia faba* infected with *Sclerotinia sclerotiorum*. This fungus produces oxalic acid, which is an important virulence factor for this and other pathogens. The failure of stomata to close in the dark was found to be due

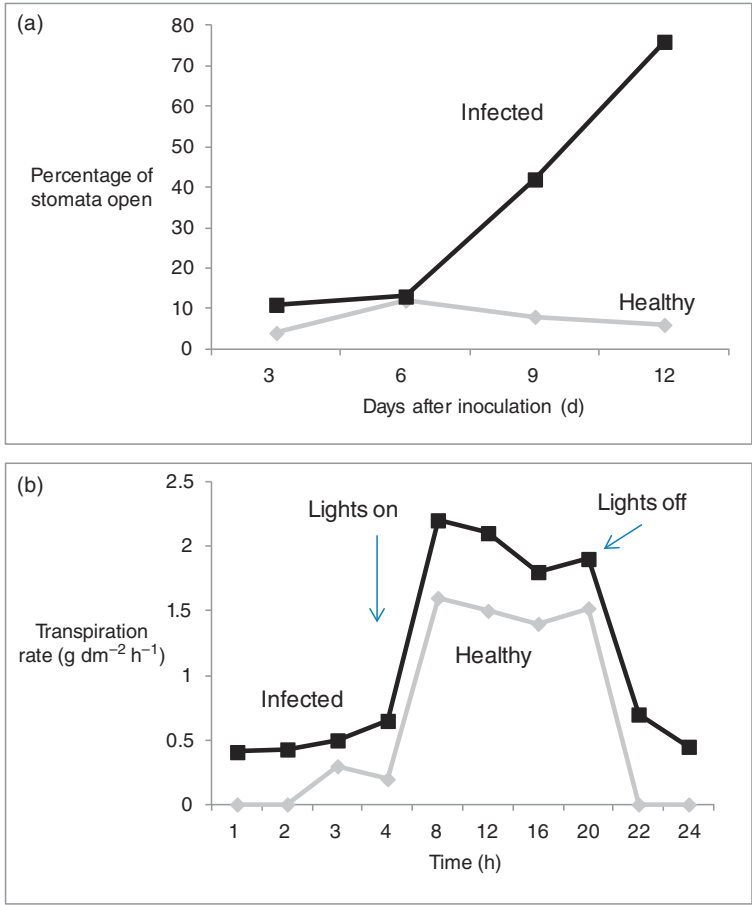


Fig. 6.2 (a) Percentage of stomata open in the dark in barley leaves, healthy and infected with *Rhynchosporium secalis*. (b) Transpiration rate of barley plants, healthy and infected with *R. secalis*, over 24 hours, including a 12-hour light period. Ayres and Jones (1975). Reproduced with permission of Elsevier.

to the accumulation of oxalic acid, which reached tissue concentrations sufficient to induce stomatal closure *in vitro* (Guimarães & Stotz, 2004). These authors found that oxalate acted via the accumulation of osmotically active molecules, thereby inducing stomatal opening and by inhibition of abscisic acid ABA-induced stomatal closure.

We saw previously that stomatal opening is reduced in compatible interactions between barley and *B. graminis* f.sp. *hordei*. However, stomatal function was also found to be impaired in incompatible interactions between barley and powdery mildew. For example, in the barley genotype P01, where resistance is based on a hypersensitive response (HR), stomata became locked open and were unable to respond to darkness or light (Fig. 6.3) or indeed to drought or exogenously supplied ABA (Prats *et al.*, 2006). Stomatal lock open was also observed in the leaves of grapevine inoculated with the oomycete pathogen, *Plasmopara viticola* (Allègre *et al.*, 2007). As might be expected, locking open of stomata in infected grapevine leaves resulted in very substantial increases in transpiration, which were not reversed by application of ABA (Fig. 6.4; Allègre *et al.*, 2007). Interestingly, when barley was inoculated with rust (*Puccinia graminis* f.sp. *hordei*), rather than stomata remaining locked open as with powdery mildew infection, they became locked shut (Prats *et al.*, 2007), highlighting interaction specific stomatal responses to pathogen challenge.

Following on from their earlier work on stomatal lock open in barley resisting powdery mildew attack via a HR (Prats *et al.*, 2006), studies were extended to include barley genotypes with different resistance alleles to *B. graminis* f.sp. *hordei*, as well as barley responding to a non-pathogen, *B. graminis* f.sp. *avenae* (oat powdery mildew) (Prats *et al.*, 2010). The different barley genotypes chosen all had resistance to powdery mildew based on HR, although the genotypes differed in their spatiotemporal patterns of HR. In all cases, inoculation with powdery mildew was associated with stomata locking open in the dark. This could have serious implications for barley crops, as stomatal lock open in the dark would increase water loss, thereby reducing grain filling in drier seasons and, ultimately, reducing grain yield (Prats *et al.*, 2010). In contrast, in barley genotypes where resistance to powdery mildew is based

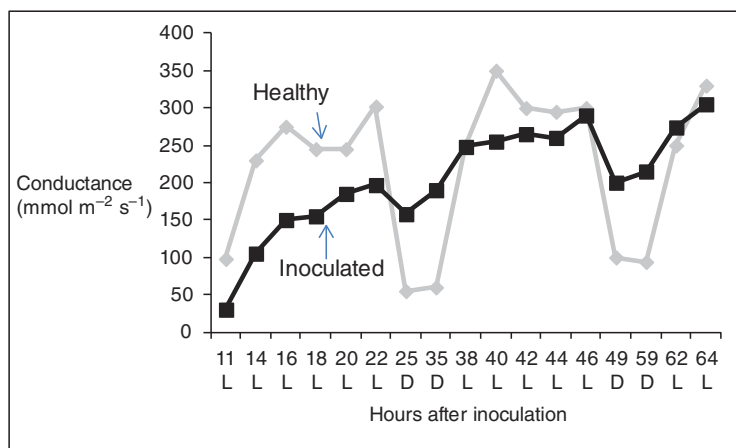


Fig. 6.3 Time-course of stomatal conductance of leaves of the resistant barley genotype P01 after inoculation with powdery mildew. Measurements were made during the light (L=light) and the dark (D=dark). Adapted from Prats *et al.* (2006). Reproduced with permission of Oxford University Press.

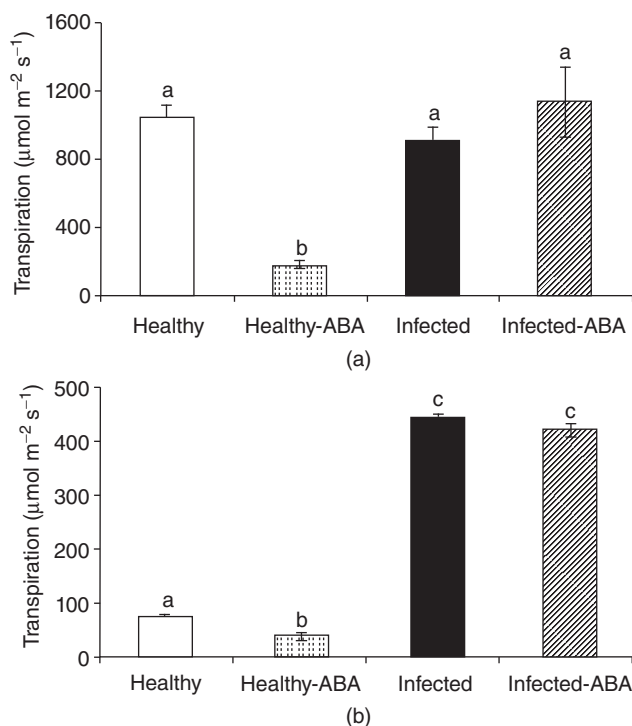


Fig. 6.4 Effect of petiolar absorption of ABA on leaf transpiration of grapevine. After ABA or water treatment (3 hours, in the light), leaf petioles were immersed in water and placed either in the light (a) or in darkness for 21 h (b), before transpiration was measured. Bars, mean $\pm$ SE ($n=4$). Values with different letters (a–c) are statistically different ($P < 0.05$). Allègre *et al.* (2007). Reproduced with permission of John Wiley & Sons.

on formation of papillae, although stomatal lock open in the dark occurs, it is transient (Prats *et al.*, 2006). The implication is that barley crops expressing papilla-based resistance would lose less water over a season than crops where mildew resistance is based on a HR. This work demonstrates that stomatal dysfunction occurs not only in compatible plant–pathogen interactions, but also in plants expressing resistance to pathogen infection, representing a hitherto unexpected cost of resistance to pathogens. However, changes in stomatal behaviour can represent more than a side effect of infection and can, in fact, play an important role in the outcome of a plant–pathogen interaction (see Box 6.1).

Box 6.1 Stomatal behaviour and plant immunity

Stomata are the most abundant pores in the leaf surface and represent a potential means of entry to the leaf for pathogens. Perhaps, it should come as no surprise therefore that during the course of evolution, stomata have acquired the ability to respond to the presence of pathogens on the leaf surface. Indeed, Melotto *et al.* (2006) found that both pathogenic and non-pathogenic bacteria on a leaf surface can promote stomatal closure

via host recognition of pathogen-associated molecular patterns (PAMPS). It appears therefore that stomata can function as part of the plant innate immune response. As might be expected, pathogens have evolved various means of overcoming this stomatal defence. Thus, the fungal pathogen *Fusicoccum amygdali* produces the toxin fusicoccin, which stimulates stomatal opening by activating a plasma membrane H^+ -ATPase (Emi *et al.*, 2001), while the bacterium *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 secretes the polyketide toxin coronatine, which can alter stomatal behaviour (Bender *et al.*, 1999). A mutant of *Pst* DC3000, which cannot produce coronatine (COR⁻), is unable to colonise the apoplast efficiently or to cause disease after inoculation (Melotto *et al.*, 2006). Later work showed that another bacterial pathogen, *Xanthomonas campestris* pv. *campestris*, was able to reverse stomatal closure induced by pathogens or by ABA via secretion of a virulence factor (Gudesblat *et al.*, 2009). The identification of host proteins capable of regulating stomatal apertures during pathogen attack provides further support for the important role of stomata in plant innate immunity (Liu *et al.*, 2009; Desclos-Theveniau *et al.*, 2012).

6.2.1.2 Damage to the leaf surface

Some pathogens, such as rust fungi, rupture the leaf surface when they sporulate. Before sporulation, rust infection is known to inhibit stomatal opening in the light. However, after sporulation, damage to the leaf surface is thought to be responsible for the increased transpiration rates commonly observed (Ayres, 1978). For example, in French bean leaves infected with *Uromyces phaseoli*, transpiration rate was reduced before sporulation but increased greatly after sporulation (Duniway & Durbin, 1971). However, large and sudden increases in transpiration are not always observed. Thus, working on barley infected with the brown rust fungus, *Puccinia hordei*, Berryman *et al.* (1991) found that although infected leaves lost their ability to maintain a favourable water status and leaf turgor and water potentials were reduced in infected leaves compared to controls, there was no sudden impact of sporulation. Nevertheless, rust-induced increases in transpiration after sporulation will exert a negative effect on leaf water relations and could result in water stress. Thus, effects on growth and yield caused by reductions in photosynthesis (see Chapter 3) could be exacerbated by rust-induced increases in transpiration.

6.2.1.3 Effects of foliar pathogens on water uptake and transport by roots

Foliar pathogens can exert profound changes in root function, including uptake and transport of water and nutrients (the latter is dealt with in Chapter 7). This should not be surprising, because infection by powdery mildews and rusts, for example, can reduce root growth and alter internal root structure, leading to reductions in the number and diameter of xylem vessels (Vizarova & Minarcic, 1974; Walters & Ayres, 1981; Tissiera & Ayres, 1988). In barley infected with powdery mildew, the volume of water moving across the whole root was reduced, although, when expressed per unit of root surface area, water movement across the root was increased (Walters & Ayres, 1982). These authors found that the hydraulic conductivity (rate of diffusion of water per unit area per unit hydrostatic pressure gradient) of the root was increased, despite a reduction in the diameter of root xylem vessels. Similar changes were found in rust-infected

groundsel (*Senecio vulgaris*), where water flux per unit of root dry weight and length was increased (Paul & Ayres, 1988), and in rusted broad bean, where Tissera and Ayres (1988) detected a stimulation on root hydraulic conductivity. Interestingly, in rust-infected broad beans, hydraulic conductivity was increased in root tissue produced after infection but not in tissue produced before infection (Tissera & Ayres, 1988). Making sweeping generalisations is usually not a good idea, especially where host–pathogen interactions are concerned. Thus, on the basis of the work described previously, it might be tempting to assume that similar changes are likely to occur in other host–rust combinations. However, in barley infected with brown rust, the root hydraulic conductivity was reduced compared to control plants (Berryman *et al.*, 1991).

6.2.2 Vascular wilt pathogens

Vascular wilt pathogens include those that enter the plant by active invasion of the root (e.g. *Fusarium* and *Verticillium* species), those entering via wounds (e.g. bacteria such as *Pseudomonas solanacearum*) and those that are introduced into aerial parts of the plant by vectors (such as the fungus *Ophiostoma ulmi*, the causal agent of Dutch Elm disease, which is carried by *Scolytus* beetles). These pathogens spread within the xylem of their hosts, and as they grow within the xylem, vessels become blocked both by the increasing biomass of the pathogen and by the polysaccharides and pectolytic enzymes they secrete. To make matters worse, the host responds by producing gums and mucilages, as well as tyloses (Fig. 6.5), which can reduce water flow through such vessels by 95%. This, in turn, can result in leaf water deficits that

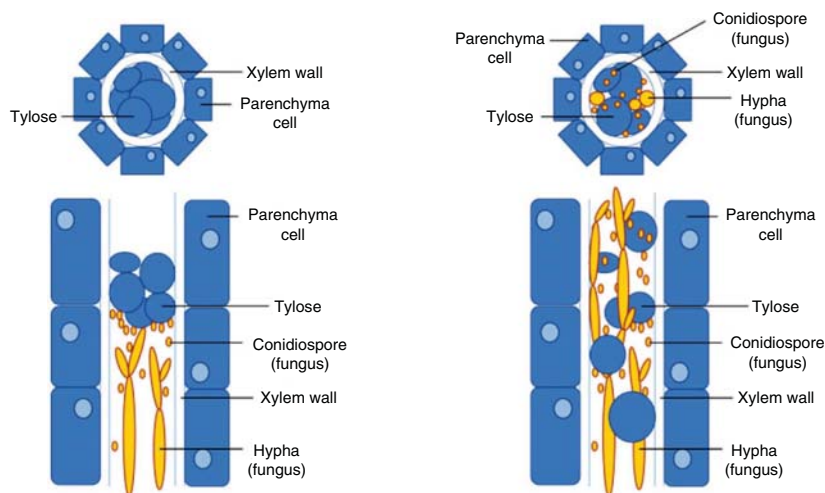


Fig. 6.5 Blockage of xylem vessels by tyloses limits pathogen growth in resistant plants but can also reduce water flow in the plant. Schematic drawing of cross-sections (a,c) or longitudinal sections (b,d) of a fungal-infected xylem vessel of a resistant (left) and a susceptible (right) plant. In the resistant plant, timely induction of the formation of tyloses, bubble-like outgrowth of the parenchyma contact cells surrounding the xylem vessels that protrude into the lumen of the vessel, are able to trap the fungus after which elimination can occur. In the susceptible plant, tylose formation cannot trap the pathogen, which is able to spread and further colonize the xylem. Yadeta and Thomma (2013). © 2013 Yadeta & Thomma/CC-BY-3.0.

can cause stomatal closure and reduce transpiration rate. For example, in field experiments on potato, infection with *Verticillium dahliae* reduced stomatal conductance and transpiration and, as a result, increased leaf temperature (Bowden & Rouse, 1991). In some plants, such as cocoa, some isolates of *V. dahliae* can induce severe defoliation, while other isolates lead to wilting, followed by desiccation of the leaves but without defoliation. Resende *et al.* (1996) found that rapid reductions in midday leaf water potential, stomatal conductance and transpiration were closely associated with the onset of foliar symptoms in *V. dahliae*-infected cocoa plants, suggesting that water stress was a major cause of symptom development. Levels of water stress were the greatest when cocoa plants were inoculated with a non-defoliating isolate of the pathogen. Interestingly, plants inoculated with a defoliating isolate of *V. dahliae* accumulated ethylene in newly developed leaves, where first symptoms usually appeared, prompting the authors to suggest that ethylene might be responsible for the accelerated leaf senescence and defoliation (Resende *et al.*, 1996).

In addition to the causes mentioned in the previous section, blockage of xylem vessels in plants infected with vascular wilt pathogens might also occur via cavitation and the subsequent formation of air embolisms. For example, Newbanks *et al.* (1983) found that embolism preceded blockage of xylem vessels by any other means in Dutch Elm disease. In contrast, in Virginia creeper (*Parthenocissus quinquefolia*) infected with the xylem-limited bacterium *Xylella fastidiosa*, reductions in water flow through the xylem were the result of vessel blockage by the pathogen and not by increased cavitation and air embolisms (McElrone *et al.*, 2003).

Toxins produced by some vascular wilt pathogens have been implicated in the altered water relations of infected hosts. In banana plants infected with *Fusarium oxysporum* f.sp. *cubense*, stomatal conductance and transpiration rate were reduced, with the result that infected plants lost less water than controls (Fig. 6.6; Dong *et al.*, 2012). However, water potentials in infected plants were reduced significantly compared to uninfected plants, suggesting an uncontrolled water loss not controlled by stomatal behaviour. This appeared to be due to an accumulation of fusaric acid, produced by *F. oxysporum* f.sp. *cubense*, the concentrations of which were positively correlated with symptom development in infected plants (Fig. 6.6; Dong *et al.*, 2012). Moreover, treatment of banana plants with fusaric acid led to remarkably similar changes in water relations to those caused by pathogen infection.

6.2.3 Root rot pathogens

Many pathogens that infect roots can cause death of root tissue, thereby reducing the surface area of root for water uptake and also disrupting the transport of water across the root. This can lead to wilting, and indeed, in plants infected with the widespread and serious pathogen *Phytophthora cinnamomi*, root tissues die, resulting in leaf yellowing, wilting and, eventually, shoot death. In chesnut saplings inoculated with *P. cinnamomi*, well-watered plants showed reduced stomatal conductance and transpiration rates, which were negatively correlated with the proportion of necrotic roots (Maurel *et al.*, 2001). However, in such plants, hydraulic conductance and leaf water potential were only affected when more than 90% of the roots were dead. Importantly, when water was restricted, infected plants showed lower stomatal conductance and transpiration than uninfected plants, irrespective of the severity of root damage, and moreover, the threshold of root damage leading to reductions in leaf water potential was lower when water was restricted (Maurel *et al.*, 2001). Earlier work by Dawson and Weste (1984)

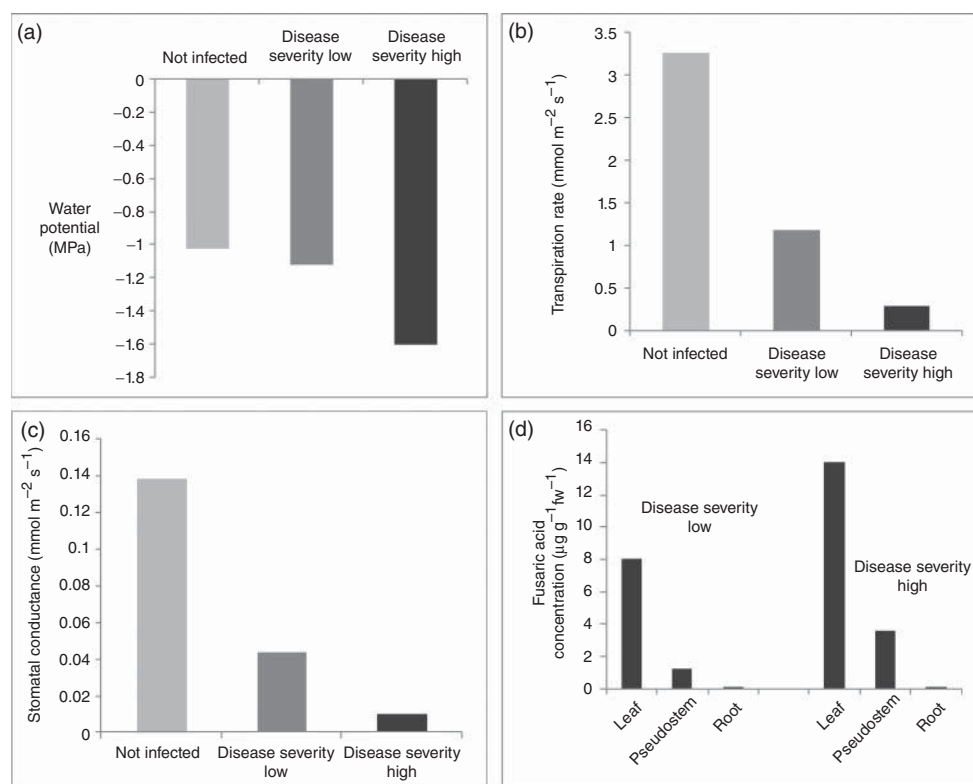


Fig. 6.6 Effects of *Fusarium oxysporum* f.sp. *cubense* infection on [a] water potential, [b] transpiration rate, [c] stomatal conductance, and [d] fusaric acid concentration, in leaves (a, b, c) and leaves, pseudostems and roots (d) of banana plants. Adapted from Dong *et al.* (2012). Reproduced with permission of Elsevier and S. Guo.

examined the development of symptoms and alterations in water relations in two *Eucalyptus* species, *E. sieberi* and *E. maculata*, susceptible and resistant, respectively, to *P. cinnamomi*. They found that in the resistant *E. maculata*, there was very little pathogen growth in the root, very limited lesion development and no other symptoms developed. In contrast, in the susceptible *E. sieberi*, there was lesion development, and wilting was observed from 3 days after inoculation. As a result, root hydraulic conductance was reduced, the effect increasing as the severity of symptoms increased. Unsurprisingly, transpiration rate was reduced in these plants, but only after the appearance of shoot symptoms. Later work demonstrated a substantial reduction in cytokinins present in the xylem sap shortly after infection by *P. cinnamomi* (Cahill *et al.*, 1986). The authors suggested that because *P. cinnamomi* preferentially penetrates and destroys root tips, which are also sites of cytokinin synthesis, this could be the reason for the dramatic reductions in cytokinins found in xylem sap of infected *E. marginata*. Reduced cytokinin concentrations have been reported in water stressed plants (see Schachtman & Goodger, 2008), leading Cahill *et al.* (1986) to suggest that an alteration in the balance of cytokinins and ABA could be the cause of the symptoms of water stress observed in *E. marginata* infected with *P. cinnamomi*.

6.3 EFFECTS OF NEMATODES ON PLANT WATER RELATIONS

Root infection by nematodes can exert considerable influence on plant water relations. Root knot nematodes invade the roots in the zone of elongation and then migrate intercellularly to the vascular cylinder, where they establish feeding sites and disrupt the vascular tissue (see Chapter 1). As a result, the water supply to the shoot is disrupted. For example, infestation with *Meloidogyne incognita* increased axial resistance to water flow and reduced total water uptake in tomato plants (Dorhout *et al.*, 1991). Similar effects were observed on tobacco plants infested with *M. incognita* and *M. javanica*, where plants extracted less water from the soil compared to non-inoculated plants (Rahi *et al.*, 1988). Such disturbances to water transport in plants attacked by root knot nematodes lead to water stress that is manifested in above-ground symptoms, such as stunting, wilting and chlorosis.

Strajnar *et al.* (2012) found that infection of tomato roots by *M. incognita* led to substantial reductions in the surface area of fine roots. Importantly, the most significant impact of infection

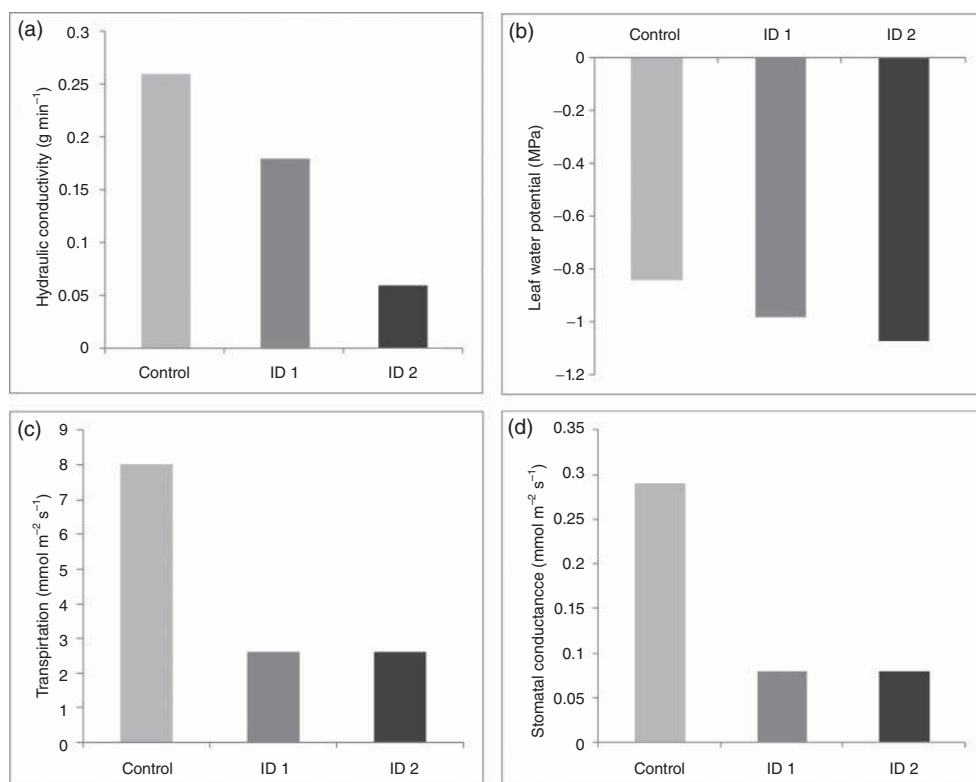


Fig. 6.7 Effect of two different inoculum densities (ID 1 and ID 2) of *Meloidogyne ethiopica* on (a) hydraulic conductivity, (b) leaf water potential, (c) transpiration rate and (d) stomatal conductance of tomato. Measurements for (a) and (b) were taken 74 days after inoculation, and for (c) and (d) 102 days after inoculation. ID 1 = 50,000 eggs per pot; ID 2 = 250,000 eggs per pot. Adapted from Strajnar *et al.* (2012). Reproduced with permission of Springer Science + Business Media

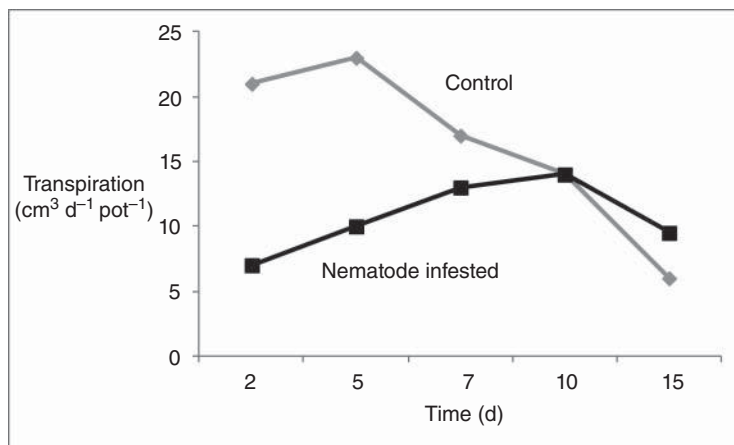


Fig. 6.8 Transpiration rates as a function of time for wheat plants grown on control soil and soil infested with 30 *Heteroda avenae* cysts per 100 g of soil. Adapted from Amir and Sinclair (1996). Reproduced with permission of Elsevier.

on host water relations occurred at this time. Thus, the hydraulic conductivity of the root system was reduced, as was stomatal conductance, transpiration and leaf water potential (Fig. 6.7; Strajnar *et al.*, 2012).

Cyst nematodes can also affect plant water relations. Thus, infection of potato roots by *Globodera pallida* resulted in reduced stomatal conductance and rates of transpiration (Schans, 1991). In this case, it was proposed that the effects on stomatal aperture were caused by a hormonal imbalance in the infected plant. Infection of wheat roots by *Heterodera avenae* also led to greatly reduced rates of transpiration. (Fig. 6.8). However, these changes were associated with reduced root growth and rooting depth, the magnitude of the reduction increasing with increasing numbers of cysts in the soil (Amir & Sinclair, 1996). Irrespective of the underlying mechanisms, cyst nematodes are clearly responsible for perturbations in stomatal behaviour and transpiration, which will ultimately influence plant growth and yield.

6.4 WATER RELATIONS IN PLANTS INFESTED WITH INSECT HERBIVORES

Attack by insect herbivores, both chewing/grazing insects and sucking/piercing insects, can lead to substantial changes in leaf gas exchange. We have already dealt with effects of insect herbivory on photosynthesis in Chapter 3, but what happens to transpiration? Chewing/grazing insects will disrupt leaf integrity, creating cut edges and abraded surfaces from which water loss will occur. Indeed, transpiration rates can be increased by such herbivory, with the magnitude of the increase dependent on the extent of leaf damage. Thus, in alder and birch, transpiration increased as the amount of leaf perforation by the alder beetle, *Agelastica alni*, increased (Fig. 6.9; Oleskyn *et al.*, 1998). Following herbivory, transpiration rates can be increased with little or no change occurring to rates of photosynthesis (Ostlie & Pedigo, 1984; Peterson & Higley, 1996). In soybean, herbivory by the Japanese beetle, *Popillia japonica*, and caterpillars

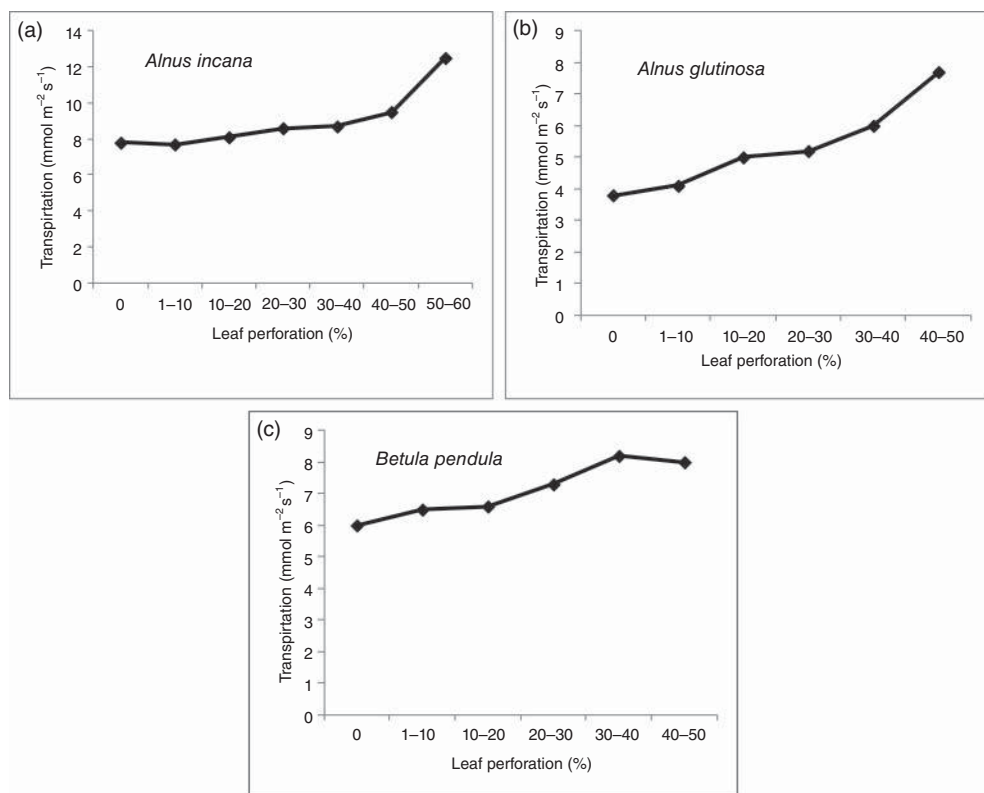


Fig. 6.9 Transpiration of (a) 40-year-old *Alnus incana*, (b) 4-year-old *A. glutinosa* and (c) 4-year-old *Betula pendula* in relation to leaf perforation by the alder beetle (*Agelastica alni*). Adapted from Oleskyn *et al.* (1998). Reproduced with permission of John Wiley & Sons.

of the corn earworm, *Helicoverpa zea*, caused increases in transpiration ranging from 20% to 90% without affecting photosynthesis (Fig. 6.10; Aldea *et al.*, 2005). Herbivory by the corn earworm led to water loss both from the damaged cuticle and from the cut edges of soybean leaves, and using a fluorescent tracer, water was found to evaporate from the apoplast approximately 100 μ m away from the cut edges of attacked leaves. Over a period of 6 days, leaves attacked by the corn earworm lost 45% more water than undamaged leaves (Aldea *et al.*, 2005). Under field conditions, such changes in water loss from damaged leaves could exacerbate water stress, with serious consequences for crop growth and yield.

Studies on the effects of the weevil, *Larinus minutus*, on growth and physiological performance of spotted knapweed, *Centaurea stoebe*, found that although this insect usually feeds on flower heads, it can feed on, and damage, stems and leaves (Wooley *et al.*, 2011). In this case, infestation by *L. minutus* led to reduced stomatal conductance and transpiration over time, and increased weevil damage was associated with decreased stomatal conductance and transpiration. Wooley *et al.* (2011) suggested that over time, the ability of spotted knapweed to compensate for herbivory decreased and that the effects of stem damage on growth and flower production were the result of the reduced stomatal conductance and rates of photosynthesis and transpiration.

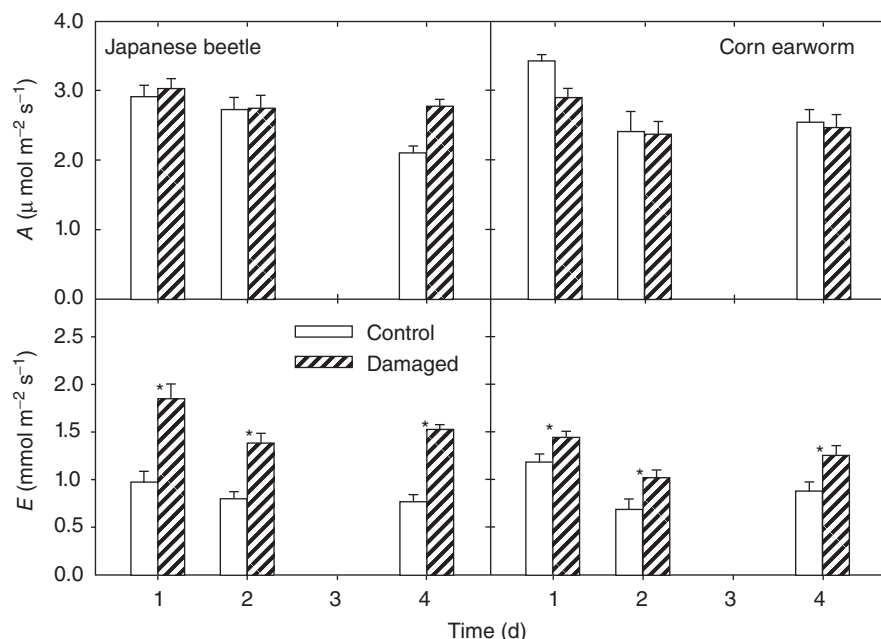


Fig. 6.10 The effects of herbivory by Japanese beetle (left) and corn earworm (right) on net photosynthesis (A) and transpiration (E) in soybean. Aldea *et al.* (2005). Reproduced with permission of John Wiley & Sons.

The potato leafhopper, *Empoasca fabae*, feeds by probing and lacerating plant cells and tissues, releasing cell contents. While the cell contents are ingested by the leafhopper, saliva is secreted into the damaged tissues. This can lead to chlorosis and wilting of leaves and can result in reduced plant growth. Infestations of *E. fabae* have been reported to result in reduced stomatal conductance and transpiration rates in various plants, including alfalfa and grapevine (Flinn *et al.*, 1990; Lenz *et al.*, 2012). It is known that *E. fabae* saliva can cause changes in tissue anatomy and function, including vascular blockage (Ecale & Backus, 1995), and Lenz *et al.* (2012) speculated that the reduced stomatal conductance observed in infested grapevine might have been due to secretion of saliva by the feeding leafhopper.

Analogous to *E. fabae*, the black bean aphid *Aphis fabae* is a piercing/sucking insect. However, whereas *E. fabae* lacerates cells, thereby releasing cell contents, the black bean aphid is a phloem feeder. Infestation of broad bean with the black bean aphid resulted in greatly increased stomatal conductance and transpiration rates, with the magnitude of these increases proportional to the level of aphid infestation (Fig. 6.11; Shannag, 2007). The mechanism responsible for the changes in stomatal conductance reported after phloem feeding by *A. fabae* is not known, but, as speculated on in the previous paragraph for the potato leafhopper (Lenz *et al.*, 2012), might be associated with secretion of saliva into host tissue (Shannag, 2007).

Leaf miners are endophagous insects that do not damage the leaf surface but rather live within the leaf by creating a structure called a mine. Larval development in the leaf miner *Phyllonorycter blancardella* is a five-stage process. The larvae feed on sap during the first

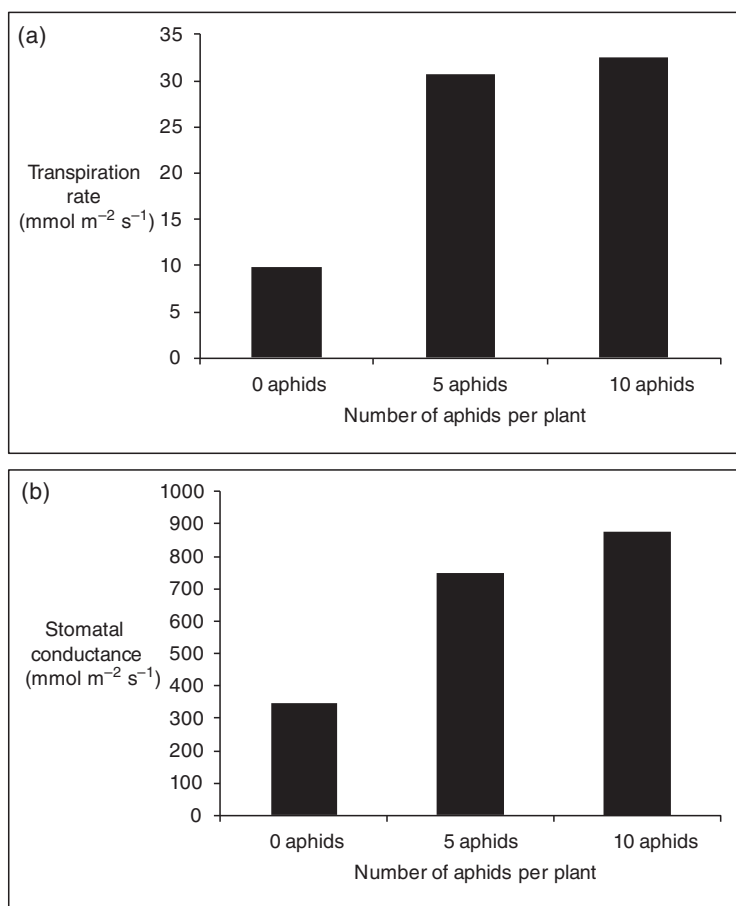


Fig. 6.11 Transpiration rate (a) and stomatal conductance (b) in faba beans infested with different numbers of the black bean aphid, *Aphis fabae*, per plant. Data from Shannag (2007).

three stages, while during the final two stages, they feed on leaf tissue. This latter feeding behaviour results in the formation of feeding windows on the upper side of the leaf; these are actually translucent patches remaining after the chlorophyll-containing tissues have been eaten (Pincebourde & Casas, 2006). The lower integument of the mine comprises very thin leaf tissue, which tends to be white but can sometimes be green. The mine protrudes from the upper leaf surface, resulting in a large air space (Fig. 6.12; Pincebourde *et al.*, 2006). When gas exchange was examined in apple leaves infested with *P. blancardella*, transpiration rates in the mine integument were reduced to a much greater extent than rates of photosynthesis (Pincebourde *et al.*, 2006). Stomatal behaviour was altered in the mined tissue, with such tissues responding differently to changes in light intensity compared to intact leaf tissue (Fig. 6.13). Thus, in mined tissues, stomatal conductance was lower at high light levels than values obtained for intact leaf tissues. The mechanism responsible for stomatal closure in

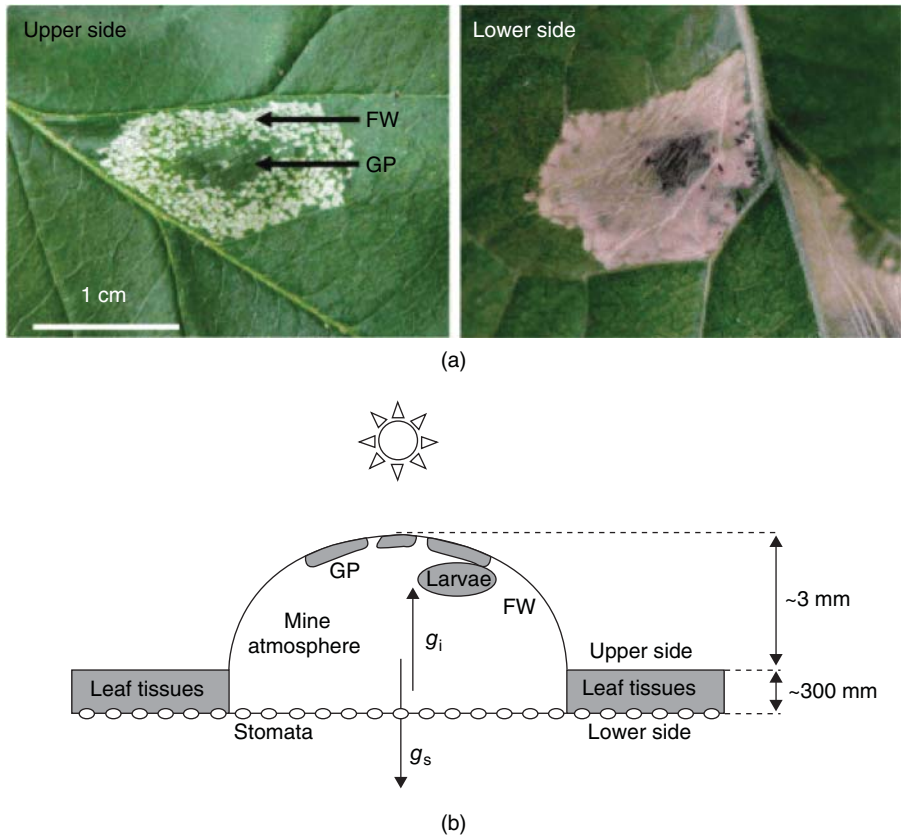


Fig. 6.12 (a) The upper (left) and lower (right) surfaces of a mine on an apple leaf caused by a larva of the leaf mining moth, *Phyllonorycter blancardella* ($\times 2.8$). The feeding windows (FW) result from the feeding activity of the larva within the mine, whereas green patches (GP) correspond to the chlorophyll-containing tissues remaining in the mine. A single mine always contains only one larva. The upper mine surface (or integument) is therefore made from both feeding windows and palisade cells (green patches). The lower mine integument corresponds to the lower, thin-translucent leaf epidermis containing stomata. The dark area in the middle of the mine corresponds to the larval dejections. The upper and the lower mine integuments are not suberized. (b) A schematic cross-section of a mine, indicating that the mine protuberance height is higher than leaf thickness, creating a very large air space inside the leaf tissues. Legend: g_s , stomatal conductance for water vapour; g_i , internal (intercellular) air space conductance for CO_2 diffusion. Pincebourde *et al.* (2006). Reproduced with permission of John Wiley & Sons.

mined tissues at moderate to high light levels is not known. However, because green patches remaining in the mines were still able to photosynthesise at rates close to those of intact leaves, while transpiration rates were much reduced, water use efficiency (WUE; the ratio of photosynthesis to transpiration) was increased (Fig. 6.14; Pincebourde *et al.*, 2006). The authors suggested that this might represent a novel mechanism by which plants might minimise losses from herbivory, that is by a trade-off between negative effects on photosynthesis and positive effects on WUE.

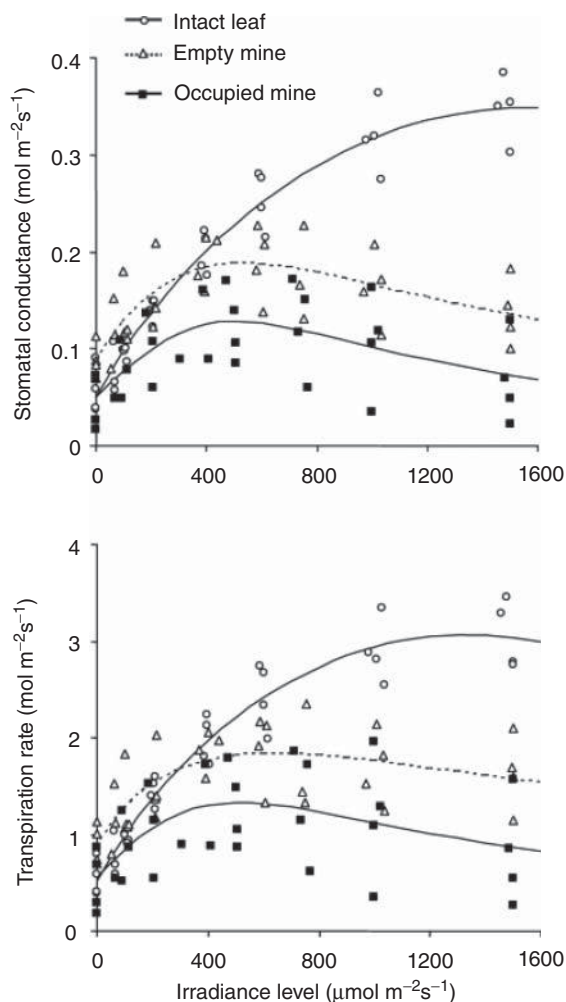


Fig. 6.13 Stomatal conductance and transpiration rate responses to irradiance level in intact leaf tissues, occupied mines and empty mines, on apple leaves infested with larvae of the leaf mining moth, *Phyllonorycter blancardella*. Pincebourde *et al.* (2006). Reproduced with permission of John Wiley & Sons.

6.5 EFFECTS OF PARASITIC ANGIOSPERMS

As we saw in Chapter 1, parasitic angiosperms can be separated into holoparasites and hemiparasites. Holoparasites lack chlorophyll and have little independent capacity to assimilate carbon and inorganic nutrients, while hemiparasites are chlorophyllous and may be obligate or facultative (Stewart & Press, 1990). Some species have functional roots (e.g. species of *Rhinanthus* and *Olax*) and are capable of taking up nutrients from the soil, and others have what appears to be a vestigial root (e.g. *Orobanchae*), while some parasitic plants have nothing that looks similar to a root or functions as a root (e.g. the Cuscutaceae and the mistletoes). The

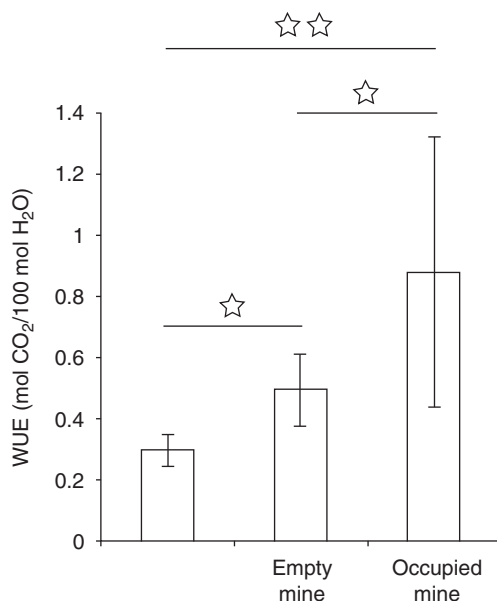


Fig. 6.14 Mean WUE in intact leaf tissues, occupied mines and empty mines on apple leaves infested with larvae of the leaf mining moth, *Phyllonorycter blancardella*. Stars indicate the level of statistical significance (one star: $P < 0.05$; two stars: $P < 0.005$). Pincebourde *et al.* (2006). Reproduced with permission of John Wiley & Sons.

movement of host resources (water and nutrients) to the parasite occurs via the haustorium, which contains both host and parasite tissue. Although there is some movement of water via direct xylem-to-xylem contact, it is small, because in many cases, direct xylem-to-xylem contacts represent just a small fraction of contact between the host and the parasite. For example, direct xylem-to-xylem contact was found in just 3% of parasite cells at the interface between the parasitic *Amyema linophyllum* (Loranthaceae) and its host, *Casuarina obesa* (Pate *et al.*, 1991). In fact, it appears that most cellular contacts at the haustorial interface with host xylem, for many parasitic plants, are with parenchyma cells (Fineran, 1987; Kuo *et al.*, 1989). Nevertheless, water movement via the haustorium from host to parasite is thought to be facilitated by lower water potential in the parasite, compared to the host, as demonstrated in the interactions between mistletoes and their hosts and the root hemiparasite *Rhinanthus serotinus* and its host (Scholander *et al.*, 1965; Klaren & van der Dijk, 1976). Transpiration rates in many hemiparasitic plants are considerably greater than those of their hosts, and indeed, this is considered to be important in maintaining the water potential gradient that exists between host and parasite (Press *et al.*, 1988). An additional factor in maintaining this gradient of water potential is the resistance to water transport caused by the haustorium, which has been quantified in host interactions with mistletoe (e.g. Glatzel, 1987; Davidson *et al.*, 1989). Later work by Ackroyd and Graves (1997) on infection of sorghum by the root hemiparasite *Striga hermonthica* showed that both a high transpiration rate in the parasite and an increased resistance across the haustorium appeared to be necessary for resources to be transferred from host to parasite.

Transpiration rates in *Striga* are considerably greater than those of its hosts (Press *et al.*, 1987; Ackroyd & Graves, 1997). Moreover, in both *S. hermonthica* and *S. asiatica*, stomatal conductance showed little response to periods of darkness or water stress, unlike their sorghum host (Press *et al.*, 1987). Transpiration rates in infected sorghum were lower than those of uninfected plants, probably reflecting the water stress induced by the very considerable loss of water to the parasite and the resulting stomatal closure. ABA arriving from the roots in the xylem stream is known to be involved in stomatal closure under water stress (e.g. Tardieu & Davies, 1992), and interestingly, increased concentrations of ABA were detected in the xylem sap of *Striga*-infected sorghum (Drennan & El Hiweris, 1979; Frost *et al.*, 1997).

Mistletoes are hemiparasitic, and some are reported to keep stomata open in an almost unregulated manner, thereby maintaining high transpiration rates even under dry conditions. The mistletoe *Viscum album*, growing on Scots pine (*Pinus sylvestris*), was found to barely regulate its stomata (Fig. 6.15), resulting in stomatal closure in the host, in an attempt to curtail its water loss and reduce the risk of hydraulic failure, at least in the short term (Zweifel *et al.*, 2012). However, despite closing its stomata, infected pine branches were not fully able to compensate for the uncontrolled water loss from the parasite.

Cuscuta campestris is an obligate holoparasite that has been studied for its potential to control the invasive perennial weed *Mikania micrantha*. Infection of *M. micrantha* by *C. campestris* was found to reduce its biomass substantially and to alter host physiology, including water relations. Shen *et al.* (2007) found that in infected plants, stomatal conductance and transpiration were considerably reduced on a diurnal basis (Fig. 6.16). The parasite also reduced rates of photosynthesis, and the authors speculated that ABA might be involved in mediating the observed changes in host physiology. Indeed, in subsequent work, ABA concentration was found to increase in infected plants, and although the increase was transient, lasting a few days, it was considered sufficient to account for the reductions in stomatal conductance, transpiration and photosynthesis (Chen *et al.*, 2011).

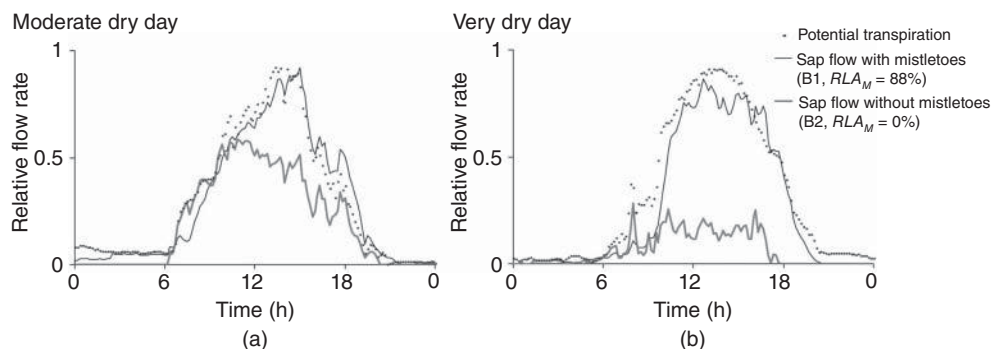


Fig. 6.15 Indication for non-regulated mistletoe stomata. (a) The measured sap flow of a branch (B1) with a very high mistletoe infection (Relative mistletoe leaf area (RLA_M) = 88%) followed potential transpiration closely, whereas the sap flow of a branch without mistletoes (B2, RLA_M = 0%) showed a strong deviation from it. (b) Under very dry conditions, the difference in sap flow between the two branches even increased, although B1 also showed a slight deviation from potential transpiration on that day. Zweifel *et al.* (2012). Reproduced with permission of Oxford University Press.

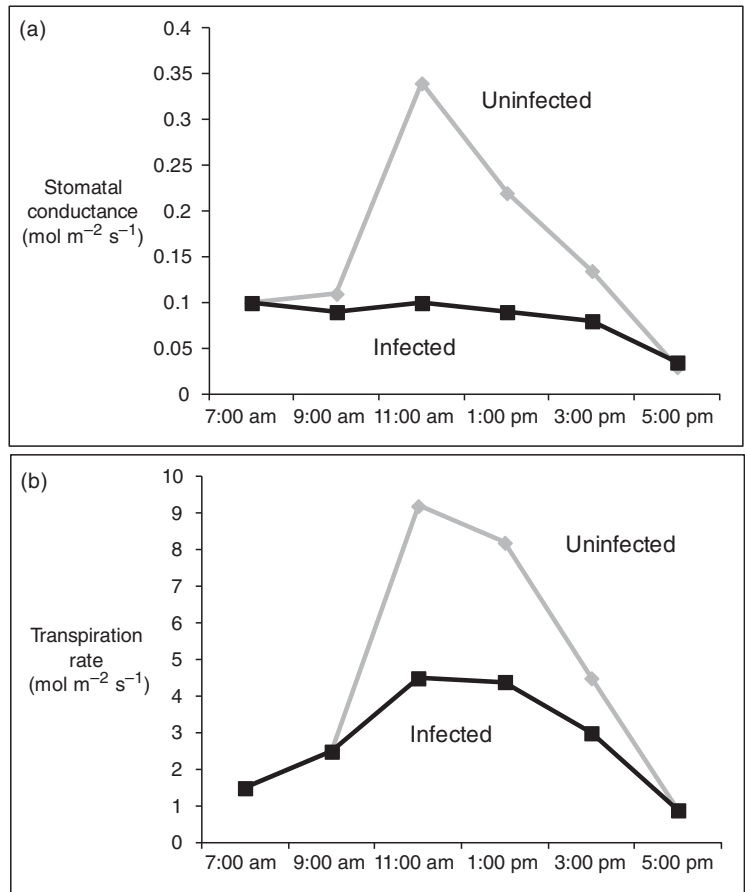


Fig. 6.16 Diurnal changes in stomatal conductance (a) and transpiration rate (b) in uninfected *Mikania micrantha* and *M. micrantha* infected with the holoparasitic plant, *Cuscuta campestris*. Adapted from Shen *et al.* (2007). Reproduced with permission of Oxford University Press.

6.6 CONCLUSIONS

Attack by pathogens, pests or parasitic plants can result in severe perturbations in plant water status. Disruptions to plant water status can be particularly badly hit when the plant loses control of stomatal behaviour. On its own, disrupted water status can cause water stress, reducing photosynthesis, plant growth and ultimately, yield. However, when combined with the other effects of attack on the host's physiology, the result can be devastating. However, plants are not always devastated by attack. The outcome depends on the intensity of attack and the level of damage inflicted and the ability of the plant to compensate for damage caused to its tissues.

But the story of plants and water does not end here. The uptake and transport of plants are closely linked to the uptake and transport of mineral nutrients, and disruptions to the former

can affect the latter. This will be the subject of the next chapter, which will examine the effects of attack on the uptake, transport and distribution of mineral nutrients in plants.

RECOMMENDED READING

- Grimmer MK, Foulkes MJ, Paveley ND, 2012. Foliar pathogenesis and plant water relations: a review. *Journal of Experimental Botany* **63**, 4321–4331.
- Zweifel R, Bangerter S, Rigling A, Sterck FJ, 2012. Pine and mistletoes: how to live with a leak in the water flow and storage system? *Journal of Experimental Botany* **63**, 2565–2578.

REFERENCES

- Akroyd RD, Graves JD, 1997. The regulation of the water potential gradient in the host and parasite relationship between *Sorghum bicolor* and *Striga hermonthica*. *Annals of Botany* **80**, 649–656.
- Aldea M, Hamilton JG, Resti JP, Zangerl AR, Berenbaum MR, DeLucia H, 2005. Indirect effects of insect herbivory on leaf gas exchange in soybean. *Plant, Cell and Environment* **28**, 402–411.
- Allègre M, Daire X, Héloir M-C, Trouvelot S, Mercier L, Adrian M, 2007. Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves. *New Phytologist* **173**, 832–840.
- Amir J, Sinclair TR, 1996. Cereal cyst nematode effects on wheat water use, and on root and shoot growth. *Field Crops Research* **47**, 13–19.
- Ayres PG, 1972. Abnormal behaviour of stomata in barley leaves infected with *Rhynchosporium secalis* (Oudem) J.J. Davis. *Journal of Experimental Botany* **23**, 683–691.
- Ayres PG, 1978. Water relations of diseased plants. In: Kozlowski TT, ed. *Water deficits and plant growth*. London: Academic Press, pp. 1–60.
- Ayres PG, Jones P, 1975. Increased transpiration and the accumulation of root absorbed ^{86}Rb in barley leaves infected by *Rhynchosporium secalis* (leaf blotch). *Physiological Plant Pathology* **7**, 49–58.
- Ayres PG, Zadoks JC, 1979. Combined effects of powdery mildew disease and soil water level on the water relations and growth of barley. *Physiological Plant Pathology* **14**, 347–361.
- Bender CL, Alarcón-Chaidez F, Gross DC, 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiology and Molecular Biology Reviews* **63**, 266–292.
- Berryman CA, Eamus D, Farrar JF, 1991. Water relations of leaves of barley infected with brown rust. *Physiological and Molecular Plant Pathology* **38**, 393–405.
- Bowden RI, Rouse DI, 1991. Effects of *Verticillium dahliae* on gas exchange of potato. *Phytopathology* **81**, 293–301.
- Cahill DM, Weste GM, Grant BR, 1986. Changes in cytokinin concentrations in xylem extrudate following infection of *Eucalyptus marginata* Donn ex Sm with *Phytophthora cinnamomi* Rands. *Plant Physiology* **81**, 1103–1109.
- Chen H, Shen H, Ye W, Cao H, Wang Z, 2011. Involvement of ABA in reduced photosynthesis and stomatal conductance in *Cuscuta campestris*-*Mikania micrantha* association. *Biologia Plantarum* **55**, 545–548.
- Davidson NJ, True KC, Pate JS, 1989. Water relations of the parasite: host relationship between the mistletoe *Amyema linophyllum* (Fenzl.) Tieghem and *Casuarina obesa* Miq. *Oecologia* **80**, 321–330.
- Dawson P, Weste G, 1984. Impact of infection by *Phytophthora cinnamomi* on the water relations of two *Eucalyptus* species that differ in susceptibility. *Phytopathology* **74**, 486–490.
- Desclos-Theveniau M, Arnaud D, Huang T-Y, Lin GJ-C, Chen W-Y, Lin Y-C, Zimmerli L, 2012. The Arabidopsis lectin receptor kinase LecRK-V.5 represses stomatal immunity induced by *Pseudomonas syringae* pv. *Tomato* DC3000. *PLoS Pathogens* **8**(2), e1002513.
- Dong X, Ling N, Wang M, Shen Q, Guo S, 2012. Fusaric acid is a crucial factor in the disturbance of leaf water imbalance in *Fusarium*-infected banana plants. *Plant Physiology and Biochemistry* **60**, 171–179.
- Dorhout R, Gommers FJ, Kolloffel C, 1991. Water transport through tomato roots infected with *Meloidogyne incognita*. *Phytopathology* **81**, 379–385.

- Drennan DHS, El Hiweris SO, 1979. Changes in growth regulating substances in *Sorghum vulgare* infected with *Striga hermonthica*. In: Musselman LJ, Worsham AD, Eplee RE, eds. *Second International Symposium on parasitic weeds*. Raleigh, USA: North Carolina State University, pp. 144–155.
- Duniway JM, Durbin RD, 1971. Some effects of *Uromyces phaseoli* on the transpiration rate and stomatal response of bean leaves. *Phytopathology* **61**, 114–119.
- Ecale CL, Backus EA, 1995. Time course of anatomical changes to stem vascular tissues of alfalfa, *Medicago sativa*, from probing injury by the potato leafhopper, *Empoasca fabae*. *Canadian Journal of Botany* **73**, 288–298.
- Emi T, Kinoshita T, Shimakazi K, 2001. Specific binding of vf14-3-3a isoform to the plasma membrane H^+ -ATPase in response to blue light and fusicoccin in guard cells of broad bean. *Plant Physiology* **125**, 1115–1125.
- Farrell GM, Preece TF, Wren MJ, 1969. Effects of infection by *Phytophthora infestans* (Mont.) de Bary on the stomata of potato leaves. *Annals of Applied Biology* **63**, 265–275.
- Fineran BA, 1987. A structural approach towards investigating transport system between host and parasite, as exemplified by some mistletoes and root parasites. In: Weber HC, Forstreuter W, eds. *Parasitic flowering plants* (Proceedings of the 4th International Symposium on Parasitic Flowering Plants). Marburg, Germany: Phillips-Universität, pp. 201–220.
- Flinn PW, Hower AA, Knievel DP, 1990. Physiological response of alfalfa to injury by *Empoasca fabae* (Homoptera: Cicadellidae). *Environmental Entomology* **19**, 176–181.
- Frost DL, Gurney AL, Press MC, Scholes JD, 1997. *Striga hermonthica* reduces photosynthesis in sorghum: The importance of stomatal limitations and a potential role for ABA? *Plant Cell and Environment* **20**, 483–492.
- Glatzel G, 1987. Haustorial resistance, foliar development and mineral nutrition in the hemiparasitic mistletoe *Loranthus europaeus* Jacq. (Loranthaceae). In: Weber HC, Forstreuter W, eds. *Parasitic flowering plants* (Proceedings of the 4th International Symposium on Parasitic Flowering Plants). Marburg, Germany: Phillips-Universität, pp. 253–262.
- Grimmer MK, Foulkes MJ, Paveley ND, 2012. Foliar pathogenesis and plant water relations: a review. *Journal of Experimental Botany* **63**, 4321–4331.
- Gudesblat GE, Torres PS, Vojnov AA, 2009. *Xanthomonas campestris* overcomes Arabidopsis stomatal innate immunity through a DSF cell-to-cell signal-regulated virulence factor. *Plant Physiology* **149**, 1017–1027.
- Guimarães RL, Stotz HU, 2004. Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection. *Plant Physiology* **136**, 3703–3711.
- Jones P, Ayres PG, 1972. The nutrition of the subcuticular mycelium of *Rhynchosporium secalis* (barley leaf blotch): permeability changes induced in the host. *Physiological Plant Pathology* **2**, 383–392.
- Klaren CH, van der Dijk S, 1976. Water relations of the hemiparasite *Rhinanthus serotinus* before and after attachment. *Physiologia Plantarum* **38**, 121–125.
- Koers S, Guzel-Deger A, Marten I, Roelfsema MRG, 2011. Barley mildew and its elicitor chitosan promote closed stomata by stimulating guard cell S-type anion channels. *The Plant Journal* **68**, 670–680.
- Kuo J, Pate JS, Davidson NJ, 1989. Ultrastructure of the haustorial interface and apoplastic continuum between host and the root hemiparasite *Oxalaphyllanthi* (Labill.) R. Br. (Olacaceae). *Protoplasma* **150**, 27–39.
- Lenz MS, Isaacs R, Flore JA, Howell GS, 2012. Photosynthetic response of Pinot gris (*Vitis vinifera* L.) grapevine leaves in response to potato leafhopper (*Empoasca fabae* Harris) infestation. *American Journal of Enology and Viticulture* **63**, 357–366.
- Liu J, Elmore JM, Fuglsang AT, Palmgren MG, Staskawicz BJ, Coaker G, 2009. RIN4 functions with plasma membrane H^+ -ATPases to regulate stomatal apertures during pathogen attack. *PLoS Biology* **7** (6), e1000139.
- Lopes DB, Berger RD, 2001. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. *Phytopathology* **91**, 212–220.
- Maurel M, Robin C, Capdeville X, Loustau D, Desprez-Loustau M-L, 2001. Effects of variable root damage caused by *Phytophthora cinnamomi* on water relations of chestnut saplings. *Annals of Forest Science* **58**, 639–651.
- McElrone AJ, Sherald JL, Forseth IN, 2003. Interactive effects of water stress and xylem-limited bacterial infection on the water relations of a host vine. *Journal of Experimental Botany* **54**, 19–430.
- Melotto M, Underwood W, Koczan J, Nomura K, He SY, 2006. Plant stomata function in innate immunity against bacterial invasion. *Cell* **126**, 969–980.

- Newbanks D, Bosch A, Zimmermann MH, 1983. Evidence for xylem dysfunction by embolization in Dutch Elm Disease. *Phytopathology* **73**, 1060–1063.
- Oleskyn J, Karolewski P, Giertych MJ, Zytowski R, Reich PB, Tjoelker MG, 1998. Primary and secondary host plants differ in leaf-level photosynthetic response to herbivory: evidence from *Alnus* and *Betula* grazed by the alder beetle, *Agelastica alni*. *New Phytologist* **140**, 239–249.
- Ostlie K, Pedigo L, 1984. Water loss from soybean after simulated and actual insect defoliation. *Environmental Entomology* **13**, 1675–1680.
- Öpik H, Rolfe S, 2005. *The physiology of flowering plants*. Cambridge: Cambridge University Press.
- Pate JS, True KC, Kuo J, 1991. Partitioning of dry matter and mineral nutrients during a reproductive cycle of the mistletoe *Amyema linophyllum* (Fenzl.) Tieghem parasitizing *Casaurina obesa* Miq. *Journal of Experimental Botany* **42**, 427–439.
- Paul ND, Ayres PG, 1988. Nutrient relations of groundsel (*Senecio vulgaris*) infected by rust (*Puccinia lagenophorae*) at a range of nutrient concentrations. II. Uptake of nitrogen, phosphorus and potassium and shoot-root interactions. *Annals of Botany* **61**, 499–506.
- Peterson RKD, Higley LG, 1996. Temporal changes in soybean gas exchange following simulated insect defoliation. *Agronomy Journal* **88**, 550–554.
- Pincebourde S, Casas J, 2006. Leaf miner-induced changes in leaf transmittance cause variations in insect respiration rates. *Journal of Insect Physiology* **52**, 194–201.
- Pincebourde S, Frak E, Sinoquet H, Regnard JL, Casas J, 2006. Herbivory mitigation through increased water-use efficiency in a leaf-mining moth-apple tree relationship. *Plant, Cell and Environment* **29**, 2238–2247.
- Prats E, Gay AP, Mur LAJ, Thomas BJ, Carver TLW, 2006. Stomatal lock-open, a consequence of epidermal cell death, follows transient suppression of stomatal opening in barley attacked by *Blumeria graminis*. *Journal of Experimental Botany* **57**, 2211–2226.
- Prats E, Carver TLW, Gay AP, Mur LAJ, 2007. Enemy at the gates. Interaction-specific stomatal responses to pathogenic challenge. *Plant Signalling & Behaviour* **2**, 275–277.
- Prats E, Gay AP, Roberts PC, Thomas BJ, Sanderson R, Paveley N, Lyngkjaer MF, Carver TLW, Mur LAJ, 2010. *Blumeria graminis* interactions with barley conditioned by different single *R* genes demonstrate a temporal and spatial relationship between stomatal dysfunction and cell death. *Phytopathology* **100**, 21–32.
- Press MC, Tuohy JM, Stewart GR, 1987. Gas exchange characteristics of the sorghum-*Striga* host parasite association. *Plant Physiology* **85**, 1143–1145.
- Press MC, Graves JD, Stewart GR, 1988. Transpiration and carbon acquisition in root hemiparasitic angiosperms. *Journal of Experimental Botany* **39**, 1009–1014.
- Rahi GS, Rich JR, Hodge C, 1988. Effect of *Meloidogyne incognita* and *M. javanica* on leaf water potential and water use of tobacco. *Journal of Nematology* **20**, 516–522.
- Resende MLV, Mepsted R, Flood J, Cooper RM, 1996. Water relations and ethylene production as related to symptom expression in cocoa seedlings infected with defoliating and non-defoliating isolates of *Verticillium dahliae*. *Plant Pathology* **45**, 964–972.
- Schachtman DP, Goodger JQD, 2008. Chemical root to shoot signalling under drought. *Trends in Plant Science* **13**, 281–287.
- Schans J, 1991. Reduction of leaf photosynthesis and transpiration rates of potato plants by second stage juveniles of *Globodera pallida*. *Plant, Cell and Environment* **14**, 707–712.
- Scholander PF, Harnall HT, Bradstreet ED, Henningsen EA, 1965. Sap pressure in vascular plants. *Science* **148**, 339–346.
- Scott P, 2008. *Physiology and behaviour of plants*. Chichester, West Sussex: John Wiley & Sons, Ltd.
- Shannag HK, 2007. Effect of black bean aphid, *Aphis fabae*, on transpiration, stomatal conductance and crude protein content of faba bean. *Annals of Applied Biology* **151**, 183–188.
- Shen H, Hong L, Ye WH, Cao HL, Wang ZM, 2007. The influence of the holoparasite *Cuscuta campestris* on the growth and photosynthesis of its host *Mikania micrantha*. *Journal of Experimental Botany* **58**, 2929–2937.
- Smith AM, Coupland G, Dolan L, Harberd N, Jones J, Martin C, Sablowski R, Amey A, 2010. *Plant biology*. New York: Garland Science.
- Stewart GR, Press MC, 1990. The physiology and biochemistry of parasitic angiosperms. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**, 127–151.
- Strajnar P, Širca S, Urek G, Šircelj H, Železnik P, Vodnik D, 2012. Effect of *Meloidogyne ethiopica* parasitism on water management and physiological stress in tomato. *European Journal of Plant Pathology* **132**, 49–57.

- Tardieu F, Davies WJ, 1992. Stomatal response to abscisic acid is a function of current plant water status. *Plant Physiology* **98**, 540–545.
- Tissera P, Ayres PG, 1988. Hydraulic conductance and anatomy of roots of *Vicia faba* plants infected by *Uromyces viciae-fabae*. *Physiological and Molecular Plant Pathology* **32**, 199–207.
- Vizarova G, Minarcic P, 1974. The influence of powdery mildew upon the cytokinins and the morphology of barley roots. *Phytopathologische Zeitschrift* **81**, 49–55.
- Walters DR, Ayres PG, 1981. Growth and branching pattern of roots of powdery mildew infected barley. *Annals of Botany* **47**, 159–162.
- Walters DR, Ayres PG, 1982. Water movement through root systems excised from healthy and mildewed barley: relationships with phosphate transport. *Physiological Plant Pathology* **20**, 275–284.
- Wilkinson S, Davies WJ, 2002. ABA-based chemical signalling: the coordination of responses to stress in plants. *Plant, Cell and Environment* **25**, 195–210.
- Wooley SC, Smith B, King C, Seastedt TR, Knochel DG, 2011. The lesser of two weevils: physiological responses of spotted knapweed (*Centaurea stoebe*) to above- and belowground herbivory by *Larinus minutus* and *Cyphocleonus achates*. *Biocontrol Science and Technology* **21**, 153–170.
- Zeyen RJ, Carver TLW, Lyngkjaer MF, 2002. Epidermal cell papillae. In: Belanger RR, Bushnell WR, Dik AJ, Carver TLW, eds. *The powdery mildews: a comprehensive treatise*. St. Paul, MN: APS Press, pp. 107–125.
- Zweifel R, Bangerter S, Rigling A, Sterck FJ, 2012. Pine and mistletoes: how to live with a leak in the water flow and storage system? *Journal of Experimental Botany* **63**, 2565–2578.

7 Mineral Nutrition in Attacked Plants

7.1 INTRODUCTION

Plants require a range of mineral nutrients for growth and development. Macronutrients are those taken up in greatest amounts and include nitrogen, phosphorous, potassium, calcium and sulphur, whereas micronutrients are taken up and used in smaller quantities and include iron, manganese and zinc. Some elements, such as silicon, although not proven to be essential for plant growth and development, are known to be beneficial to plants (Daroub & Snyder, 2007). Uptake and transport of these minerals in plants can be greatly affected by interactions with pathogens, pests and parasitic plants. However, before we look at the effects of attack by these organisms on mineral uptake and transport, let us first reacquaint ourselves briefly with the mechanisms by which plants take up some of the macronutrients from the soil and what happens to them after uptake.

Plants absorb nitrogen in their ionic forms, ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). In well-aerated soils, ammonium is rapidly converted to nitrite and then to nitrate, which means that most nitrogen taken up by plants is in the form of nitrate. Nitrate is taken up into root epidermal and cortical cells via nitrate transporters. These are located on the plasma membrane of root cells (these are also found on the plasma membrane of leaf cells) and are nitrate–proton co-transporters, which act in concert with the plasma membrane ATP-driven proton pump (Fig. 7.1; Smith *et al.*, 2010). There are two families of nitrate transporters, NRT1 and NRT2; the former is expressed mainly in roots and possesses both low and high affinity uptake, while the latter has a very high affinity for nitrate and is regulated both developmentally and diurnally (Smith *et al.*, 2010). Plants can also take up ammonium, using ammonium transporters, some of which are induced when nitrogen levels in the soil are low, whereas others are induced by the presence of ammonium. Once in the plant, ammonium is rapidly converted to nitrite and then nitrate, by nitrite reductase and nitrate reductase, respectively. Nitrate is then converted to glutamate and glutamine by the enzymes glutamine synthetase (GS) and glutamine:2-oxoglutarate aminotransferase (GOGAT) (Fig. 7.2; Smith *et al.*, 2010). How much nitrate assimilation occurs in the root depends on the plant species, with many trees and shrubs assimilating nitrate mostly in the roots, while many herbaceous species assimilate

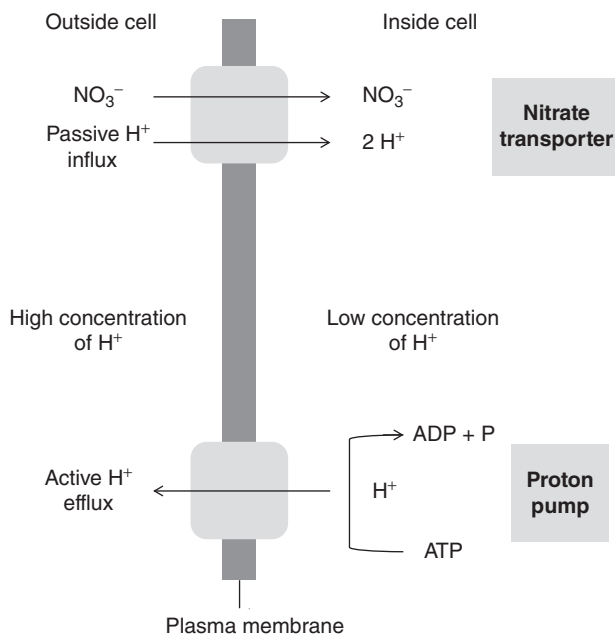


Fig. 7.1 Transport of nitrate into plant cells. Protons are exported from the cell by a proton pump in a process driven by the hydrolysis of ATP. Proton influx back into the cell occurs via the nitrate transporter and is coupled to nitrate import. Smith *et al.* 2010. Reproduced with permission of Taylor & Francis.

nitrate primarily in the leaves. Where nitrate is assimilated in the plant is influenced by its availability, with assimilation occurring in the roots when nitrate supply is limited and taking place in leaves when nitrate supply is plentiful. The amount of nitrate reductase protein and the activity of the enzyme are controlled by many factors, including nitrate abundance, light levels, diurnal and circadian cycles and concentrations of sucrose and glutamine. Diurnal changes also occur in the activities of GS and GOGAT. In fact, diurnal changes in nitrate assimilation coordinate nitrate assimilation with diurnal patterns of carbon assimilation and export in leaves, as well as with photorespiration (Smith *et al.*, 2010).

Phosphorus in the form of phosphate is essential for many functions in the plant, and although it can reach relatively high levels in plant tissues, making up about 0.2% of plant dry weight, its availability in the soil is extremely limited. This is because most soil phosphate is strongly adsorbed to soil particles, making it insoluble. Because the level of soluble phosphate in soils is low, its uptake by roots creates a zone 1–5 mm from the root surface, which is depleted of phosphate. The availability of phosphate to the plant can be increased by the secretion of organic anions such as citrate by root epidermal cells. This acidifies the area immediately surrounding the root, thereby liberating bound phosphate from soil particles. Root epidermal cells can also secrete an enzyme called acid phosphatase, which reacts with organic phosphorus containing compounds in the soil, releasing free phosphate ions. Plants can also obtain phosphate from soil via the symbiotic association of their roots with mycorrhizal fungi. Hyphae of mycorrhizal fungi can exploit a much larger volume of soil than plant roots on their own, and much of the phosphate they take up is transferred to the plant root cells. The roots take

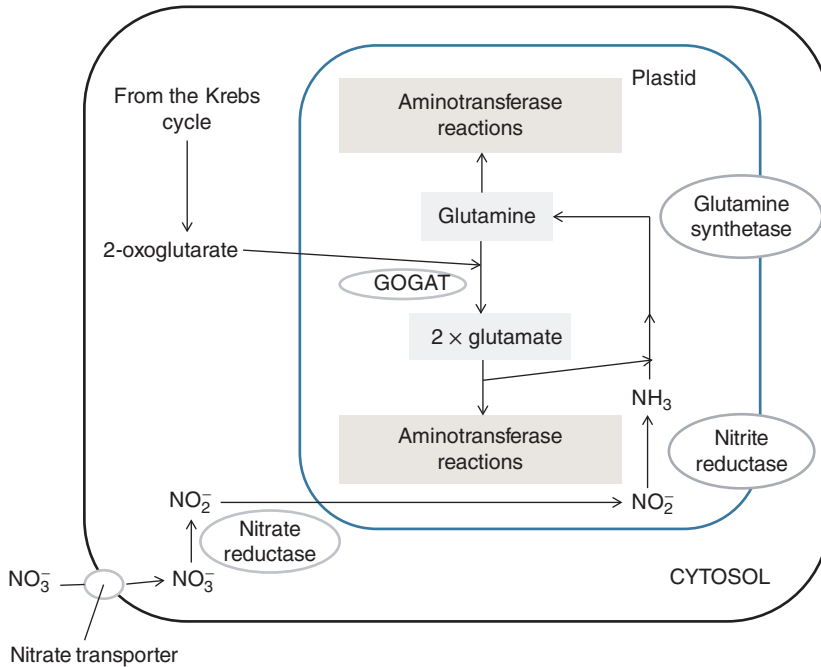


Fig. 7.2 Overview of nitrogen assimilation. Nitrate is transported into the cell via a nitrate transporter, whereupon it is reduced to nitrite in to cytosol, followed by reduction to ammonium (shown as NH_3 in the figure) in the plastid. The nitrogen is then transferred to amino acids via the activities of glutamine synthetase and glutamine: 2-oxoglutarate aminotransferase (GOGAT), using 2-oxoglutarate from the Krebs cycle. Nitrogen in glutamine and glutamate is then used in the biosynthesis of other nitrogen-containing compounds via aminotransferase reactions. Smith *et al.* 2010. Reproduced with permission of Taylor & Francis.

up phosphate into the cells using high affinity phosphate transporters, which import phosphate in exchange for protons in a process driven by H^+ -ATPases. The phosphate is then rapidly incorporated into organic molecules or is transported around the plant to tissues where it is required.

Sulphur is present in the soil mostly as sulphate (SO_4^{2-}), in which form it is taken up by plants. Uptake occurs via sulphate transporters, which exchange sulphate for protons. Once in the plant, sulphate is assimilated, the first step being reduction to sulphite (SO_3^{2-}), followed by reduction to sulphide (S^{2-}) and then transfer to an amino acid backbone to form cysteine. Although some sulphate is assimilated in root cells, most is transported in the xylem to leaves for assimilation. Transport of assimilated sulphur from leaves in the phloem is usually in the form of glutathione.

Finally, potassium is taken up by plant roots as the K^+ cation. Passive diffusion of potassium into root cells occurs in response to a potassium concentration gradient, via root ionophores (membrane-bound proteins that act as channels for specific cations). Uptake can also be mediated via active transport, where ATP activates a membrane-bound proton pump. This pump ejects H^+ from the cell, establishing an electrochemical gradient favouring the entry of both H^+ and potassium back into the cell (Rice, 2007).

Once mineral ions have entered root cells, they move between cells by diffusion through plasmodesmata. After crossing the Casparian strip and endodermis, they are pumped into the apoplast between the cells of the stele, from whence they are moved in the xylem to other parts of the plant.

7.2 MINERAL NUTRITION IN PLANT–PATHOGEN INTERACTIONS

Pathogen infection can result in severe disruption to nutrient uptake and translocation and, ultimately, to concentrations of the particular minerals in plant tissues. However, as we will see in the following sections, the mechanisms responsible for any changes in tissue concentrations of nutrients will depend on the extent to which the nutrient is metabolised (if at all), the plant organ infected (root, leaves, stems and reproductive parts) and the particular host–pathogen interaction.

7.2.1 Effects of foliar pathogens

The amount of any nutrient in a leaf represents a balance between import in the xylem and export in the phloem. It stands to reason therefore that should this equilibrium be disrupted, the level of that nutrient in the leaf will change. We saw in Chapter 5 that in leaves infected with biotrophic fungi such as powdery mildews and rusts, translocation of assimilates from the leaf is reduced. In Chapter 6, we discovered that infection by such pathogens, especially rusts, increases transpiration, thereby increasing xylem flux to the leaf. This suggests that nutrient levels should increase in leaves infected with biotrophic fungal pathogens, and indeed, this has been demonstrated in a number of host–pathogen interactions. For example, the concentrations of a number of cations (e.g. magnesium and potassium) increased in leaves of barley infected with brown rust, due primarily, at least in the case of potassium, to a reduction in export from the leaf in the phloem, rather than increased import into the leaf in the xylem (Fig. 7.3; Ahmad *et al.*, 1982). Levels of phosphorus also increased in rust-infected leaves, apparently as a result of reduced re-translocation coupled with active accumulation within the infected leaf. This is interesting, as there are many reports of solute accumulation at sites of fungal infection on such leaves, such as accumulation of phosphorus (P^{32}) at infection sites in *Phaseolus vulgaris* infected with the rust, *Uromyces phaseoli* (Gerwitz & Durbin, 1965).

The minerals discussed in the previous section must be taken up by roots, and it is reasonable to ask whether foliar infection by rusts or powdery mildews affects root uptake of these minerals. Interestingly, and as we have seen on many previous occasions, what happens depends on the particular host–pathogen system. For example, Ahmad *et al.* (1982) found that the uptake of various cations was increased in rusted barley plants, while phosphorus uptake was unaffected by infection. In contrast, phosphorus uptake and its subsequent translocation to the shoot were increased in barley infected with powdery mildew (Walters & Ayres, 1981). In addition, the uptake of potassium and chloride ions, but not sodium, was increased in mildew-infected barley plants (Walters, 1981).

From our previous discussions, the role, if any, of increased transpiration rates in altering nutrient concentrations in infected leaves is not clear. Indeed, the accumulation of P^{32} in rust-infected bean leaves was evident some 4–5 days before any increase in transpiration was detected (Gerwitz & Durbin, 1965). However, a clear role for transpiration in accounting for

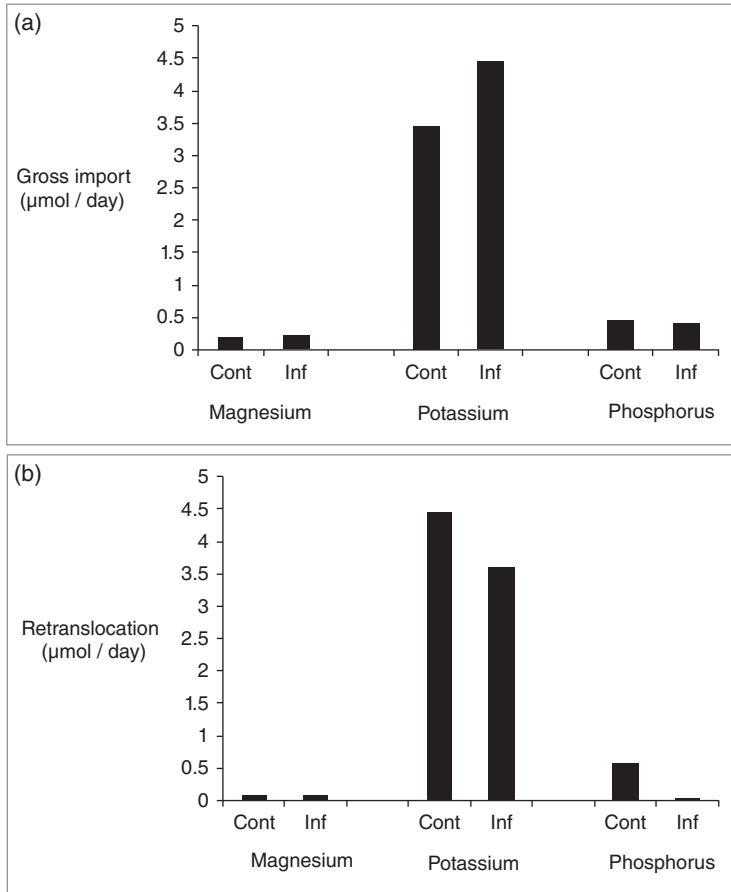


Fig. 7.3 Gross fluxes into (a), and re-translocation from (b), control and brown rust-infected barley leaves, for magnesium, potassium and phosphorus. Data from Ahmad *et al.* 1982.

increased nutrient concentrations in infected leaves was provided by Ayres & Jones (1975). Working on barley infected with the hemibiotrophic fungal pathogen *Rhynchosporium secalis* (now *Rhynchosporium commune*), they found increased accumulation of Rb^{86} (rubidium is not distinguished from potassium by most plants and was used instead of potassium in uptake studies) occurring at the same time and place as increases in transpiration. Moreover, accumulation of Rb^{86} was reduced when rates of transpiration were reduced (Fig. 7.4; Ayres & Jones, 1975), providing clear evidence, in this host–pathogen system, of a role for increased transpiration in accumulation of a solute in infected leaves.

So far, we have not mentioned nitrogen, which undergoes considerable metabolism after uptake. Changes in nitrogen uptake and subsequent metabolism in plants infected with obligately biotrophic fungal pathogens depend, once again, on the particular interaction. Thus, while nitrate content was reduced in roots and leaves of barley infected with powdery mildew (Walters & Ayres, 1980; Murray & Ayres, 1986), it was not altered in leaves of wheat and rye infected with rust (Rohringer, 1957; Piening, 1972) and was increased in leek leaves infected

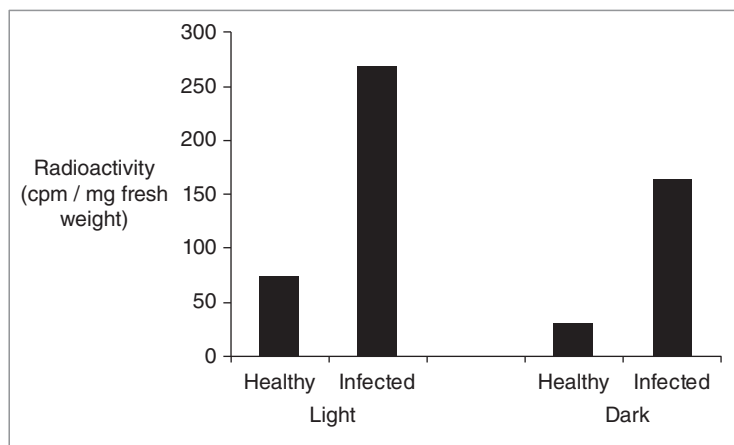


Fig. 7.4 Effect of illumination on the accumulation of root absorbed ^{86}Rb in healthy and *Rhynchosporium*-infected barley leaves. Data from Ayres & Jones 1975.

with rust (Roberts & Walters, 1988). In barley infected with brown rust, despite an increase in nitrate uptake by roots, total nitrogen content was lower than uninfected controls (Ahmad *et al.*, 1982). According to the authors, this might have reflected the substantial loss of nitrogen from infected leaves in fungal spores, which were shown to lose some 55% of nitrogen imported into the leaf.

It is worth spending a bit more time on the barley–powdery mildew interaction, as several studies have examined nitrogen uptake and metabolism in detail in this system. The reduced nitrate content of roots of powdery-mildew-infected barley plants appeared to be the result of reduced nitrate uptake by roots (Walters & Ayres, 1980; Murray & Ayres, 1986). Since nitrate uptake was reported to be sensitive to energy supply (Mengel & Viro, 1978), it was suggested that the reduced nitrate uptake in mildewed barley might have been the result of the reduced translocation of assimilates from powdery-mildew-infected leaves to roots (Fric, 1975; Walters & Ayres, 1982). Interestingly, rates of nitrogen reduction by both roots and shoots were altered by infection (Walters & Ayres, 1980; Murray & Ayres, 1986). Before sporulation of the fungus, nitrate reduction by the shoots was not affected by infection, although nitrate reduction in roots was reduced. In contrast, after sporulation, nitrate reduction in the shoots stopped but increased in the roots (Fig. 7.5; Murray & Ayres, 1986). During sporulation, mildew infection stopped the recycling of nitrogen from the shoots to the roots. Since recycled nitrogen from the shoot usually provides roots of barley with a large proportion of its requirement for nitrogen, the increased rate of nitrate reduction by roots of mildewed plants after sporulation might have represented a response to compensate for the absence of a supply of recycled nitrogen from the shoot (Murray & Ayres, 1986).

Although nitrate levels are reduced in mildewed barley, levels of ammonium increase in both leaves and roots (Sadler & Scott, 1974; Walters & Ayres, 1980). While the increased ammonium in mildewed leaves probably represented an increased breakdown of amino acids and amides (Sadler & Scott, 1974), in roots of mildewed plants, it was probably the result of a greatly decreased assimilation of ammonium via both the GS–GOGAT and glutamate dehydrogenase pathways (Walters & Ayres, 1980). Interestingly, the activity of asparagine

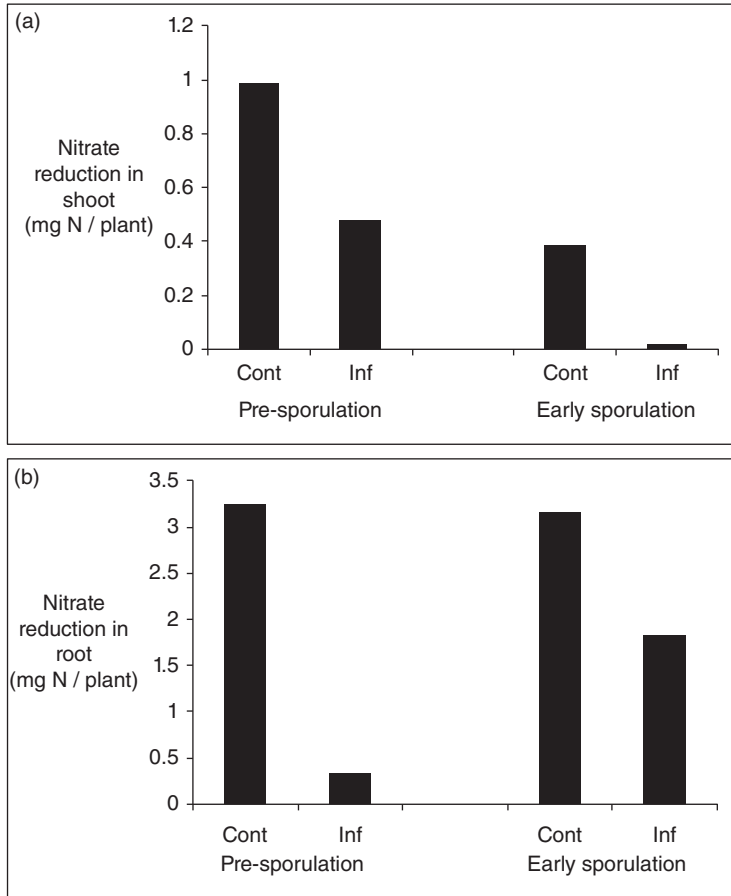


Fig. 7.5 Calculated rates of reduction of nitrate in shoots (a) and roots (b) of healthy and powdery-mildew-infected barley, pre-sporulation and during early sporulation. Values are expressed as mg N per plant over a 4-day period. Data from Murray & Ayres 1986.

synthetase (AS) was increased in leaves and roots of mildewed barley (Sadler & Scott, 1974; Walters & Ayres, 1980). This enzyme can synthesise asparagine using ammonium and aspartate, and although its K_m for ammonium is high, it should be active if ammonium levels are sufficiently high (Givan, 1979). When carbohydrates are plentiful and ATP levels are high, conditions will favour operation of the GS–GOGAT pathway but inhibit the activity of AS (Lea & Fowden, 1975). In mildewed barley, and particularly in roots of infected plants, where carbohydrate supply is reduced, conditions are likely to favour the activity of AS. It was suggested that the increased AS activity might represent an attempt to detoxify the accumulated ammonium ions, which are highly toxic and capable of disrupting electron transfer (Walters & Ayres, 1980; Walters, 1985).

Before we leave foliar pathogens, it is worth having a look at sulphur. A hallmark of biotrophy is the loss of key genes required to assimilate sulphur, something that has been found in a number of biotroph pathogens, including the oomycete *Hyaloperonospora arabidopsidis*, the

powdery mildew fungus *Blumeria graminis* and the black stem rust fungus *Puccinia graminis* f.sp. *tritici* (Baxter *et al.*, 2010; Spanu *et al.*, 2010; Duplessis *et al.*, 2011). In the poplar rust fungus, *Melampsora larici-populina*, although genes required to perform primary sulphur assimilation were detected, a transketolase domain of the beta-subunit of the sulphide reductase was missing (Duplessis *et al.*, 2011). When poplar was inoculated with virulent or avirulent strains of *M. larici-populina*, there was substantial induction of a host sulphate transporter (Petre *et al.*, 2012), tempting the authors to speculate that perhaps the pathogen uses target manipulation of poplar physiology in order to obtain sulphate. It is interesting to note that this sulphate transporter was also induced by pre-symbiotic contact of poplar roots with a symbiotic fungus and might represent a conserved mechanism targeted by symbiotic and pathogenic biotrophs to achieve compatibility with their poplar host (Petre *et al.*, 2012).

7.2.2 Effects of root-infecting pathogens

So far, we have looked at the effects of foliar pathogens on mineral uptake and concentrations. What about effects of root-infecting pathogens? Alterations in mineral uptake and translocation might be expected if pathogens destroy root tissue or disrupt xylem function. Indeed, Hornby & Fitt (1981) divided cereal root-infecting fungi into three groups on the basis of their effects on root function: (i) pathogens that do not penetrate the stele of the root or affect root function or shoot growth, such as *Phialophora radicola*; (ii) pathogens that damage the stele, thereby disrupting root function and shoot growth, such as *Gaeumannomyces graminis*; and (iii) pathogens that exert an effect on shoot growth before the root stele or root function are affected, such as *Fusarium culmorum*.

Early work on wheat infected by *G. graminis* suggested that effects on host physiology were due to disruption of water uptake by roots (Asher, 1972). However, subsequent research demonstrated the importance of pathogen invasion and disruption of the host phloem in determining effects on host mineral uptake (Clarkson *et al.*, 1975). These workers found that xylem translocation was little affected in infected roots that were still elongating but was severely decreased where phloem translocation and root elongation had stopped due to disruption of the phloem by *G. graminis*. Later work by Fitt & Hornby (1978) showed that 1 week after planting wheat plants into sand containing *G. graminis*, assimilates could not be detected in some seminal roots, presumably due to phloem disruption. In this case, shoot mineral content was not affected, because there were enough undamaged roots to supply shoots with minerals. However, the situation changed by 5 weeks after planting, when there were no undamaged roots, and assimilates were only accumulating in undamaged crown roots. In this case, because of severe phloem disruption, control of ion uptake broke down in diseased roots. The failure of assimilates to reach sites of uptake in damaged roots meant that uptake of potassium, which is mainly active, was decreased, leading to reduced concentrations in shoots (Fig. 7.6; Fitt & Hornby, 1978). In contrast, calcium contents of shoots of infected plants increased (Fig. 7.6), because it is immobile in the phloem and cannot be redistributed from senescent leaves. Not surprisingly, perhaps, nitrate uptake by wheat root segments below take-all lesions was reduced (Schoeny *et al.*, 2003), as uptake of nitrate is also energy dependent. Interestingly, in severely infected plants, nitrate uptake per unit of efficient root was increased, possibly as a compensatory response to satisfy shoot nitrogen demand (Schoeny *et al.*, 2003). What do these changes mean for plants under field conditions? Well, studies by Macdonald & Gutteridge (2012) found that severe take-all infection of winter wheat significantly decreased the capacity of the crop to take up nitrogen, reducing recovery of nitrogen fertiliser by some 37% of that applied to

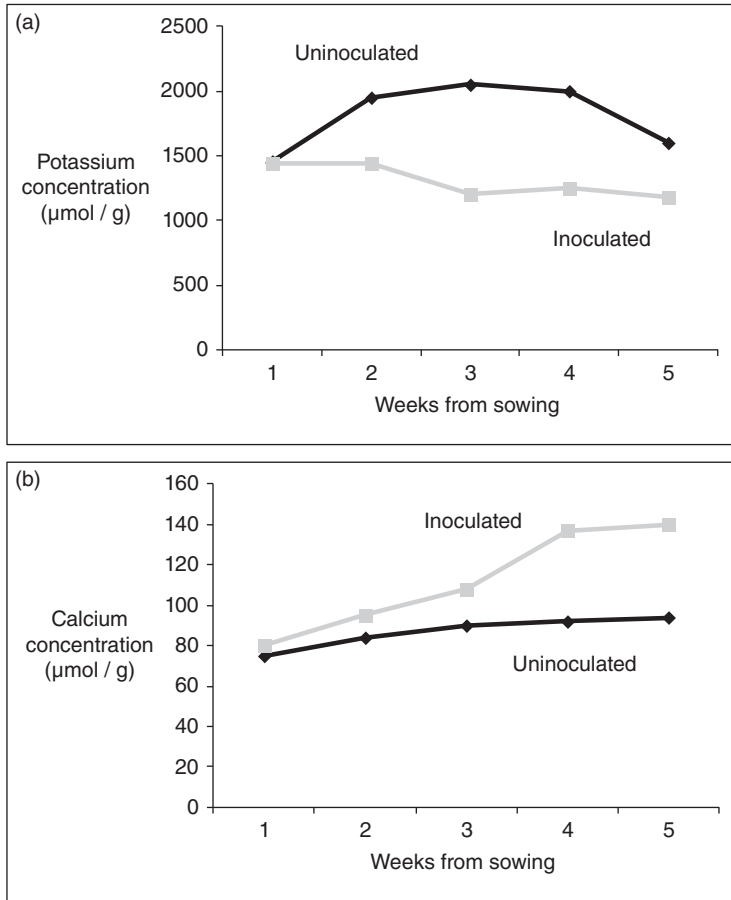


Fig. 7.6 Concentrations of potassium (a) and calcium (b) in shoots of uninoculated wheat plants and plants inoculated with the take-all pathogen, *Gaemannomyces graminis*. Adapted from Fitt & Hornby 1978. Reproduced with permission of Elsevier.

the crop. As a result, severe take-all increased the risk of nitrate leaching from patches land supporting severe take-all infection.

Fitt & Hornby (1978) also found that shoot potassium content in wheat decreased just 1 week after planting into sand infested with *F. culmorum*, although the pathogen did not penetrate the root stele until some 3–4 weeks later. It was suspected that pathogen-produced toxins might have been responsible for this rapid effect, and indeed, it is now known that toxin production is important in the pathogenesis of *F. culmorum* on barley seedlings (Hestbjerg *et al.*, 2002). Many *Fusarium* species produce the phytotoxin fusaric acid and work on fusaric acid isolated from *Fusarium oxysporum* f.sp. *niveum*, which infects watermelon, found that it disrupted nitrogen metabolism in the host. Thus, watermelon plants treated with fusaric acid exhibited greatly decreased uptake of ammonium, resulting in a decline in total leaf nitrogen content (Wu *et al.*, 2007). There was enhanced activity of proteolytic enzymes, leading to protein degradation and decreased content of amides as a result of an inhibition of AS activity.

Moreover, activities of enzymes involved in amino acid biosynthesis (GS, GOGAT and GDH) were also suppressed by treatment with fusaric acid. In short, treatment of watermelon plants with fusaric acid led to a complete collapse of nitrogen metabolism (Wu *et al.*, 2007).

7.2.3 Nitrogen metabolism and plant defence against pathogens

7.2.3.1 Slash and burn: nitrogen mobilisation as a defence strategy?

Pathogens infect plants in order to obtain assimilates and nutrients to enable them to complete their life cycles. One of the major nutrients sought by invading pathogens is nitrogen. It has been suggested that once pathogens enter the plant, they encounter a nitrogen-limiting environment (Divon *et al.*, 2006), and indeed, a number of fungal pathogenicity genes appear to be controlled by nitrogen starvation and might be dependent on nitrogen response transcription factors (Snoeiijers *et al.*, 2000; Thomma *et al.*, 2006). However, whether all pathogens encounter a nitrogen-limiting environment *in planta* and, indeed, to what extent such an environment affects pathogenicity are still unclear. For example, nitrate non-utilising mutants of *Magnaporthe grisea* did not exhibit any reduction in pathogenicity, suggesting that nitrate is not essential for this fungal pathogen *in planta* (Lau & Hamer, 1996). The effect of nitrogen on pathogen development and disease severity appears to depend, at least in part, on its lifestyle. Although nitrogen fertilisation can enhance plant defence, it will also provide nitrogenous compounds that would be available for pathogen uptake. However, such increased pools of nitrogenous material in plant cells are likely to be of benefit to biotrophs and hemibiotrophs rather than necrotrophs, which can access nitrogen from the breakdown of host cells.

As we have seen in previous chapters, pathogen infection often results in profound changes in host source–sink relationships. It has been suggested that these changes mimic those in source–sink relationships that occur during senescence (Masclaux *et al.*, 2000), and indeed, the expression of plant genes involved in nutrient recycling, proteolysis and amino acid transport, for example, is enhanced by biotic stress (AbuQamar *et al.*, 2006). Interestingly, several genes involved in nitrogen remobilisation, including GS, GDH and AS, are up-regulated by pathogen infection (e.g. Pérez-García *et al.*, 1995, 1998; Olea *et al.*, 2004; Pageau *et al.*, 2006). Angiosperms are known to contain two isoforms of GS, a cytosolic enzyme (GS1) and an isoenzyme located in the chloroplast (GS2). In tomato leaves infected with the bacterial pathogen *Pseudomonas syringae*, chloroplasts degenerate and GS2 is down-regulated, while GS1 is up-regulated (Pérez-García *et al.*, 1995). It was suggested that up-regulation of GS1 would ensure the assimilation of ammonium released from chloroplast degeneration, and the glutamine formed could then be used to transport nitrogen out of the infected leaf towards healthy plant tissues. Subsequent work found enhanced expression of a gene encoding AS in tomato leaves infected with *P. syringae*, suggesting that in this system, the combined up-regulation of GS1 and AS represents a route for transferring ammonium released from protein breakdown into asparagine for transport out of infected leaves (Olea *et al.*, 2004). Similar results for GS1 and GS2 were obtained in *P. vulgaris* infected with *Colletotrichum lindemuthianum*, and it was suggested that remobilisation of nitrogen in infected leaves could be considered as part of a slash-and-burn strategy, the aim of which is to deprive the pathogen of important nutrients (Tavernier *et al.*, 2007). *C. lindemuthianum* is a hemibiotroph, with an initial biotrophic phase lasting up to 5 days, during which time, it uses nutrients in the apoplast of the host leaf. The transition to necrotrophy occurs 6 days after inoculation, possibly because

apoplastic nutrients are no longer able to support fungal development and necrotrophy would make greater nutrient concentrations available to the fungus. It is interesting to note that in this host–pathogen system, glutamine accumulation occurs 6 days after inoculation, just as the fungus is switching from biotrophy to necrotrophy, leading Tavernier *et al.* (2007) to suggest that perhaps glutamine is involved in crosstalk between host and parasite. Interestingly, a rapid (5 h after inoculation) and strong induction of the *ASI* gene in pepper was shown to be essential for resistance against the hemibiotrophic *Xanthomonas campestris* pv. *vesicatoria*, whereas later (15–20 h after inoculation) induction of *ASI* was associated with susceptibility (Hwang *et al.*, 2011). These results highlight the importance of timing in the slash-and-burn defence strategy.

Whether the slash-and-burn approach of remobilising nitrogen reflects a useful defence strategy might be questioned if the pathogen is able to switch from biotrophy to necrotrophy, thereby enabling it to access previously unavailable nutrient sources. But what if a pathogen is unable to make this lifestyle switch? Well, the biotrophic fungal pathogen *Ustilago maydis* appears to have a different solution to this problem of nutrient limitation. This fungus can infect all aerial parts of the maize plant, resulting in the formation of tumours as a consequence of host cell hypertrophy. On leaves, these tumours can span the entire leaf blade. These tumours were found to be strong sinks for nitrogen, with organic nitrogen being imported from systemic source leaves to tumour tissue. It appears therefore that *U. maydis* overcomes nitrogen limitation in infected leaves by inducing tumours, thus generating a strong sink, capable of out-competing other systemic sink tissues in the infected plant (Horst *et al.*, 2010).

As hinted previously, just how effective the slash-and-burn strategy is in defence appears dependent on the lifestyle of the pathogen. For some necrotrophs, such as *Botrytis cinerea*, induction of senescence is a mode of pathogenicity (Swartzberg *et al.*, 2008), suggesting that the opposite strategy, that is translocation of nitrogen towards the infected area in order to supply resources to challenged host cells, could be useful for host defence (Seifi *et al.*, 2013a). Several lines of evidence lend support to this suggestion. For example, overexpression of glutamate transporters in *Arabidopsis* increased ammonium transport within challenged cells, leading to delayed senescence and increased resistance to *B. cinerea*, while *Arabidopsis* overexpressing arginase, an urea-generating enzyme supplying the GS/GOGAT cycle with ammonium, was found to express increased resistance to *B. cinerea* (Kang *et al.*, 2006; Brauc *et al.*, 2012).

7.2.3.2 Glutamate metabolism and plant disease: endurance and evasion

Programmed cell death, such as the hypersensitive response (HR), is mainly controlled via perturbations in cellular redox balances through the generation of reactive oxygen species (ROS) (Van Doorn *et al.*, 2011). Since glutamate metabolism involves complex ROS generating and scavenging machinery, it could be considered important in determining host redox status during pathogen infection (Seifi *et al.*, 2013a). It is worth reminding ourselves that while ROS-triggered HR might be effective against biotrophs and hemibiotrophs, some necrotrophs might favour, or even induce, ROS-mediated cell death as part of their invasion strategy (e.g. Van Baarlen *et al.*, 2004). In contrast, maintaining host cell viability, through, for example, the ROS-scavenging capacity of the GS/GOGAT pathway, could be an effective defence against necrotrophic pathogens, although such a strategy might favour biotrophic pathogens. Indeed, inhibiting the GS/GOGAT pathway, thereby reducing the supply of

glutamine from the apoplast into the cytosol, resulted in enhanced resistance to the biotrophic pathogen *Erysiphe cichoracearum* and the hemibiotrophic *Colletotrichum higginsianum* (Liu *et al.*, 2010). Interestingly, continued activity of the GS/GOGAT pathway, mediated by overactivation of cytosolic GS1, was a feature of the early stages of the compatible interaction between tomato and *P. syringae* (Perez-Garcia *et al.*, 1998a, b). Seifi *et al.* (2013a) suggest that the GS/GOGAT cycle could be considered a molecular on/off switch of cell viability, negatively controlling resistance against biotrophs and hemibiotrophs but positively controlling resistance against necrotrophs. This hypothesis has received support from recent work on the *sitiens* mutant of tomato (which is deficient in ABA), where the ability of this mutant to resist the necrotrophic *B. cinerea* was correlated with its ability to maintain activity of the GS/GOGAT cycle (Seifi *et al.*, 2013b).

Plant defence is costly, requiring huge amounts of energy and intermediates generated by the tricarboxylic acid (TCA) cycle. Under such circumstances, replenishing the TCA cycle is crucial, and this is achieved, in part, by the GABA (γ -aminobutyric acid) shunt. This is a cytosolic-mitochondrial pathway that connects the GS/GOGAT cycle to the TCA cycle. The first step in this shunt is the formation of GABA using glutamate as the main substrate. It is possible therefore that the ability of the GABA shunt to replenish the TCA cycle requires a constant level of cytosolic glutamate produced via the GS/GOGAT cycle, once more highlighting the role of glutamate metabolism in plant defence (Seifi *et al.*, 2013a). Interestingly, overexpression of cytosolic aspartate transaminase in *Arabidopsis* increased susceptibility to *B. cinerea*, probably through overconsumption of the cytosolic glutamate pool, thereby depleting metabolites such as GABA (Brauc *et al.*, 2011). Indeed, it appears that exhausting the TCA cycle could be an effective virulence strategy for some necrotrophic pathogens (Seifi *et al.*, 2013a).

Seifi *et al.* (2013a) have proposed a model describing the two main functionalities of host glutamate metabolism – evasion and endurance – in response to infection by biotrophs, hemibiotrophs and necrotrophs. They view reconfiguration of host glutamate metabolism during pathogen attack as a double-edged sword, either promoting the defensive strategy of the host or being exploited by the pathogen.

7.3 MINERAL NUTRITION IN PLANT-NEMATODE INTERACTIONS

As we saw in Chapter 2, plant parasitic nematodes can have a considerable impact on plant growth. Their effects on the plant are the result, in large part, of their feeding activity, and the uptake of nutrients leads to a continuous loss of water and nutrients from plant tissue. The physiological changes induced in the plant are not only restricted to infected tissues, but can also affect tissues remote from the site of attack. Sedentary nematodes, such as cyst and root-knot nematodes, have evolved sophisticated interactions with their hosts, where affected host cells, as well as surrounding tissues and even the entire plant, can be modified in order to provide nutrients to the parasite. Cyst nematodes induce syncytial feeding structures in the host, whereas root-knot nematodes cause the formation of giant cells, which are embedded in root galls (Chapter 1). These changes enable the nematodes to withdraw large amounts of water and solutes from the host. In the process, a metabolic sink is induced in the infected root, due either to the feeding activity of the nematodes or to a sophisticated manipulation of

host cells by the nematodes. Whatever the mechanism underlying formation of the sink, the amount of material taken up by adult females of *Heterodera schachtii* from *Arabidopsis* roots was four times the volume of the syncytium (Sijmons *et al.*, 1991). Considerable accumulation of carbohydrates such as sucrose can occur in syncytia compared to control roots (Hofmann *et al.*, 2007), and greatly elevated levels of amino acids were also found in these structures (Grundler *et al.*, 1991).

In syncytia and giant cells, protuberances of the cell wall are formed at the interface with the xylem, suggesting an exchange of water and inorganic solutes (Jones & Northcote, 1972). Substantial amounts of water and solutes are transported from the xylem, through these cell wall ingrowths, probably via water channels, facilitating passage across membranes (Gheysen & Fenoll, 2002). Differential expression of aquaporin genes has been reported in *Arabidopsis thaliana* infected with root-knot nematodes, with some genes up-regulated and others down-regulated (Jammes *et al.*, 2005). This fine regulation of the transcriptional control of aquaporin genes in nematode feeding cells might be related to the various functions proposed for aquaporin proteins, such as growth control, water transport and osmoregulation (Maurel & Chrispeels, 2001).

As we saw in Chapter 2, nematodes can markedly affect root growth. In the field, potato crops infected by potato-cyst nematodes generally exhibit reduced root growth several weeks after planting. However, because shoot growth tends to be reduced more than root growth, shoot: root ratio is reduced (Evans, 1982; Trudgill & Cotes, 1983). This suggests that potato-cyst nematodes reduce root elongation and also impair the physiological functioning of the roots. Indeed, crops infected by potato-cyst nematodes tend to have reduced foliar concentrations of nitrogen, phosphorus and potassium (Trudgill *et al.*, 1975a,b; Evans & Franco, 1979; de Ruijter & Haverkort, 1999), and phosphorus uptake per unit length of potato roots is reduced by nematode infection (de Ruijter & Haverkort, 1999). However, the latter authors found an interaction with soil pH, with potato-cyst nematodes reducing tuber yield by 19% when soil pH was 4.5, while yield was reduced by 44% at a soil pH of 6.5. The increased yield loss at the higher pH was explained by an effect of both soil pH and nematodes on the uptake of phosphorus (de Ruijter & Haverkort, 1999).

Some nematodes, such as root-knot nematodes (*Meloidogyne* spp.) are known to occur in legume nodules and have been reported to reduce nitrogen fixation (Barker & Hussey, 1976), although there are reports of both increases and reductions in nitrogen fixation by root-knot nematodes in soybean (Baldwin *et al.*, 1979). The effect of nematodes on nitrogen fixation depends on various factors, including soil conditions, nematode species and the species of legume under investigation. For example, nitrogen fixation was reduced significantly in *Lablab purpureus* infected with *Meloidogyne* spp. but was not significantly affected in another legume, *Mucuna pruriens* (Ibewiro *et al.*, 2000). This difference might have been due, in part, to the greater numbers of the nematode found in roots of *L. purpureus* compared to *M. pruriens*.

7.4 MINERAL NUTRITION IN PLANT-INSECT INTERACTIONS

Despite the ubiquity of herbivory, plants survive because they possess a formidable array of defences to ward off insect attackers (Walters, 2010). However, plants still suffer tissue loss as a result of herbivory, because insects have evolved mechanisms to minimise the impact of

plant defences. The fact that plants survive tissue loss due to herbivory, is due, in part, to their ability to tolerate such losses, allowing them to maintain fitness after tissue damage. Of particular interest to this chapter is the ability of plants to reallocate resources after attack. We have already seen in Chapter 5 that within hours of foliar herbivory, plants can reallocate newly formed carbon away from the site of attack (Babst *et al.*, 2005; Schwachtje *et al.*, 2006). But what happens to, for example, nitrogen? Frost & Hunter (2008) found that damage to leaves of oak seedlings by larvae of the white-marked tussock moth (*Orgyia leucostigma*) stimulated allocation of nitrogen away from fine roots and towards tap roots, while new stem tissue from herbivore-damaged seedlings accumulated new nitrogen more rapidly than did controls. The authors suggested that this represented increased storage of newly assimilated nitrogen following herbivory. Interestingly, this differs from the situation in an annual grass, where simulated herbivory (clipping leaves) led to an increase in levels of foliar nitrogen (Hamilton & Frank, 2001). These differences between oak seedlings and an annual grass might reflect those in life

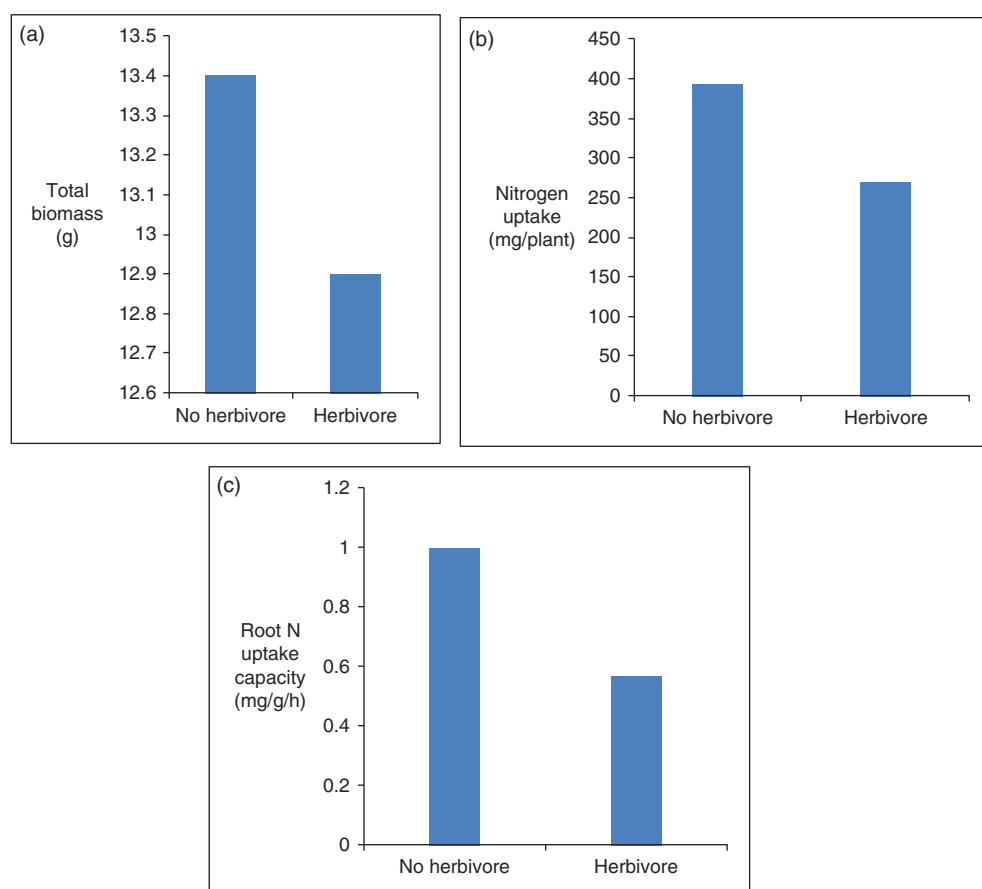


Fig. 7.7 Total biomass (a), whole plant nitrogen uptake (b) and root nitrogen uptake capacity (c) of *Centaurea maculosa* to root herbivory. Data from Newingham *et al.* 2007.

history. Thus, although it might be useful for an annual grass to reinvest new nitrogen immediately into shoot growth, it might be more appropriate for long-lived oak seedlings to store nitrogen for future growth (Frost & Hunter, 2008).

So what happens to allocation of nitrogen after root herbivory? Newingham *et al.* (2007) examined this in *Centaurea maculosa*, an invasive North American plant species, which shows a high degree of tolerance to the root-boring herbivore, *Agapeta zoegana*. They found that root herbivory reduced nitrogen uptake by the whole plant by more than 30% and root uptake capacity by 50% (Fig. 7.7). Despite this reduction in nitrogen uptake, root herbivory had no effect on root biomass (Fig. 7.7) or shoot nitrogen status. Interestingly, attacked plants maintained shoot nitrogen status by allocating more of the acquired nitrogen from the root to the shoot (Fig. 7.8). Newingham *et al.* (2007) suggest that this provides a useful mechanism for the plant to overcome the devastating effect of a herbivore-induced nitrogen deficit. In subsequent

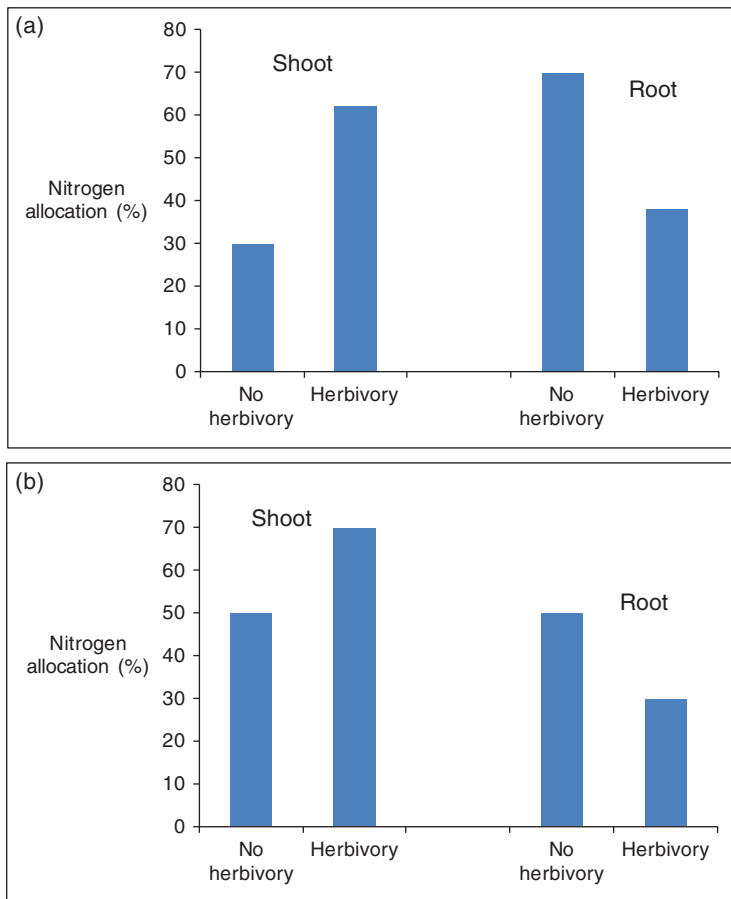


Fig. 7.8 The effect of root herbivory on (a) short-term nitrogen allocation and (b) whole-season nitrogen allocation between roots and shoots in *Centaurea maculosa*. Adapted from Newingham *et al.* 2007. Reproduced with permission from Springer Science + Business Media and B. Newingham.

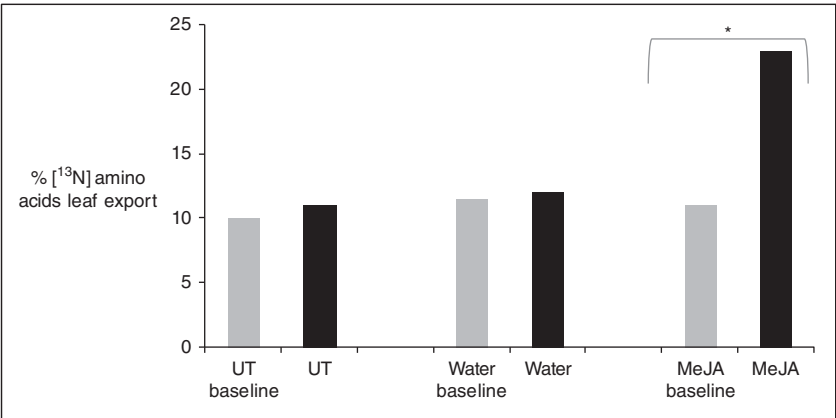


Fig. 7.9 Percentage of newly acquired nitrogen exported by tomato leaf 3 before treatment (baseline – grey bars) and after treatment with either water or methyl jasmonate (MeJA) (black bars). Some plants received no treatment (UT). * = treatment with MeJA was significantly different from plants before treatment (MeJA baseline). Gómez, 2010. Reproduced with permission from John Wiley & Sons.

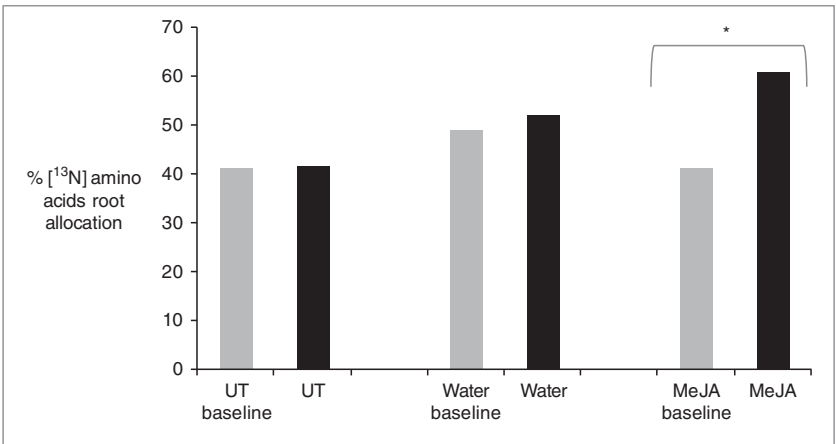


Fig. 7.10 Percentage of newly acquired nitrogen allocated to tomato roots before treatment (baseline – grey bars) and after treatment with either water or methyl jasmonate (MeJA) (black bars). Some plants received no treatment (UT). * = treatment with MeJA was significantly different from plants prior to treatment (MeJA baseline). Gómez, 2010. Reproduced with permission from John Wiley & Sons.

research, Gómez *et al.* (2010) used methyl jasmonate (MeJA) to imulate foliar herbivory on tomato. They found a rapid increase in export of newly formed amino acids out of leaves in response to MeJA treatment (Fig. 7.9), together with a large increase in root allocation of newly synthesised amino acids (Fig. 7.10). Gómez *et al.* (2010) suggest that this rapid reallocation of nitrogen from leaves to roots following simulated foliar herbivory would reduce the chance

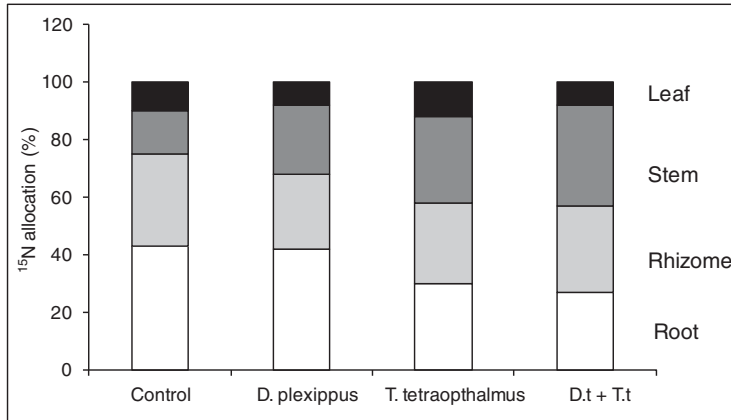


Fig. 7.11 The effects of damage by *Danaus plexipus* and *Tetraopes tetraopthalmus* on the allocation of ^{15}N among tissues of *Asclepias syriaca*. Adapted from Tao & Hunter 2013. Reproduced with permission of Springer Science + Business Media.

of resources being lost to herbivores, thereby increasing the potential for plant regrowth and survival after insect attack.

In nature, plants are likely to have to deal with multiple attackers simultaneously. With this in mind, Tao & Hunter (2013) examined the effects on common milkweed (*Asclepias syriaca*) of attack by both an above-ground herbivore (the monarch caterpillar, *Danaus plexipus*) and a below-ground herbivore (larvae of the red milkweed beetle, *Tetraopes tetraopthalmus*). The workers found that milkweed increased allocation of new nitrogen to stems at the expense of allocation to damaged tissues (roots or leaves). When plants were attacked by both herbivores simultaneously, allocation of resources to stems was greater than that induced by either herbivore alone (Fig. 7.11). As suggested by Tao & Hunter (2011), by preferentially transporting nitrogen away from sites of attack, plants can both protect their nitrogen from consumption by insects and reduce their nutritional value and appeal to insect herbivores.

The studies described previously have examined allocation patterns of nitrogen within herbivore-attacked plants. What happens to nitrogen fluxes out of plant roots after herbivory? It is known that both foliar and root herbivory can alter the exudation of carbon from roots, which in turn can affect nitrogen availability in the soil (e.g. Hamilton & Frank, 2001; Ayres *et al.*, 2004). The effects of herbivory on nitrogen fluxes from plant roots were studied by Ayres *et al.* (2007), who subjected white clover (*Trifolium repens*) to defoliation (by clipping) and root herbivory (not by an insect, but by the obligate root-feeding nematode, *Heterodera trifolii*). They found that defoliation not only increased root biomass and shoot production, but also led to a fivefold increase in nitrogen, derived from clover, in roots of perennial ryegrass, *Lolium perenne* (Fig. 7.12). In contrast, root herbivory by the nematode resulted in a slight reduction in nitrogen transfer from clover to ryegrass. The finding that defoliation of a common grassland legume can greatly increase transfer of its nitrogen to neighbouring plants by directly affecting nitrogen fluxes from roots is important, as it could alter competitive interactions, with knock-on consequences for plant community structure (Ayres *et al.*, 2007).

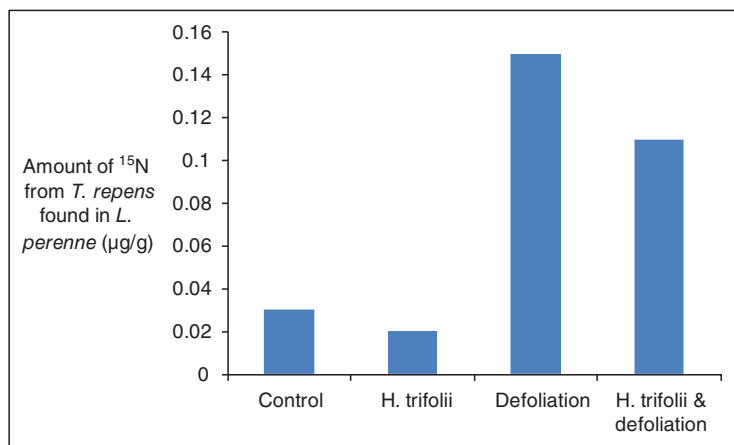


Fig. 7.12 Effect of the root herbivore *Heterodera trifolii* and defoliation on *Trifolium repens*-derived excess ^{15}N concentration in *Lolium perenne* roots per unit excess ^{15}N in *T. repens* roots. Adapted from Ayres *et al.* (2007). Reproduced with permission of John Wiley & Sons.

7.5 MINERAL NUTRITION IN INTERACTIONS BETWEEN PLANTS AND PARASITIC ANGIOSPERMS

The transfer of host solutes into a parasitic plant from its host depends on the formation of a bridge between the two organisms (Chapter 1). This bridge, the haustorium, can be formed within various host tissues and has led to distinctions being made between ‘shoot parasites’ and ‘root parasites’. However, such descriptions say nothing about the mechanisms by which parasitic plants attach to their hosts, how they obtain solutes from the host or their impact on nutrition of the host. Most parasitic plants make contact with the vascular system of the host, with some forming xylem-to-xylem contact with direct luminal contact between host and parasite, and others forming phloem-to-phloem contact, and others where solute transfer occurs via specialised transfer cells (Hibberd & Jeschke, 2001).

Carbon compounds form a large proportion of the solutes transferred from host to parasite (Chapter 5), and among the other solutes, nitrogen is a major constituent of transferred material. *Cuscuta reflexa* is a holoparasitic angiosperm, which attaches itself to the stem of its hosts and whose haustoria access host phloem via specific transfer cells (Dörr, 1972) and host xylem via tracheids (e.g. Bäuml *et al.*, 1993). When attached to its host, it represents an incredibly powerful sink, diverting the major portion of host assimilates to itself and in the process, reducing growth of the host plant (Jeschke *et al.*, 1994; Jeschke & Hilpert, 1997; see also Chapters 2 and 5). In the legume *Lupinus albus* parasitized by *C. reflexa*, competition by the parasite for nitrogen was severe, with the parasite incorporating nitrogen equalling 223% of current fixation by the host, despite the fact that host nitrogen fixation was severely depressed (Jeschke *et al.*, 1994). Withdrawal of nitrogen from the host phloem led to severe losses of nitrogen from host leaves and roots and substantial reductions in nitrogen concentrations. This required very considerable xylem-to-phloem transfer of nitrogen, because the xylem, as the major supply route for nitrogen, was not exploited substantially by *C. reflexa* (Jeschke *et al.*,

1994). The results suggest that *C. reflexa* affected its host adversely by depriving it mainly of its nitrogen. It appears that the changes in nitrogen metabolism in *C. reflexa* were the result of the considerable sink effect created by the parasite, leading to a huge reduction in assimilate supply to the nodulated host root (Jeschke *et al.*, 1994). On a non-nitrogen-fixing host, such as *Ricinus communis*, infection by *C. reflexa* led to rather different changes in nitrogen metabolism (Jeschke & Hilpert, 1997). In this case, tissue nitrogen levels were increased in the host, and nitrate uptake per unit of root weight was increased in infected plants. The latter was reflected in the nitrate concentration in the xylem sap of infected plants, which was greatly increased. In contrast, amino acid concentrations in the xylem sap were reduced in parasitized plants, suggesting that assimilation of nitrate was inhibited in roots of parasitized plants (Jeschke & Hilpert, 1997). This *Cuscuta*-induced inhibition of nitrate assimilation in the roots and increased nitrate uptake suggest that nitrate reduction is shifted to the shoot in plants infected with *C. reflexa* (Fig. 7.13; Jeschke & Hilpert, 1997).

Nitrogen metabolism was also affected in tobacco infected with the phloem-tapping parasite *Orobancha cernua*. In this case, nitrogen uptake by the infected plant was increased, while the concentration of amino acids in the xylem sap of infected plants was greatly reduced compared to uninfected plants (Fig. 7.14; Hibberd *et al.*, 1999). The concentration of most amino acids in the xylem sap of *O. cernua* was higher than that collected from infected plants, although

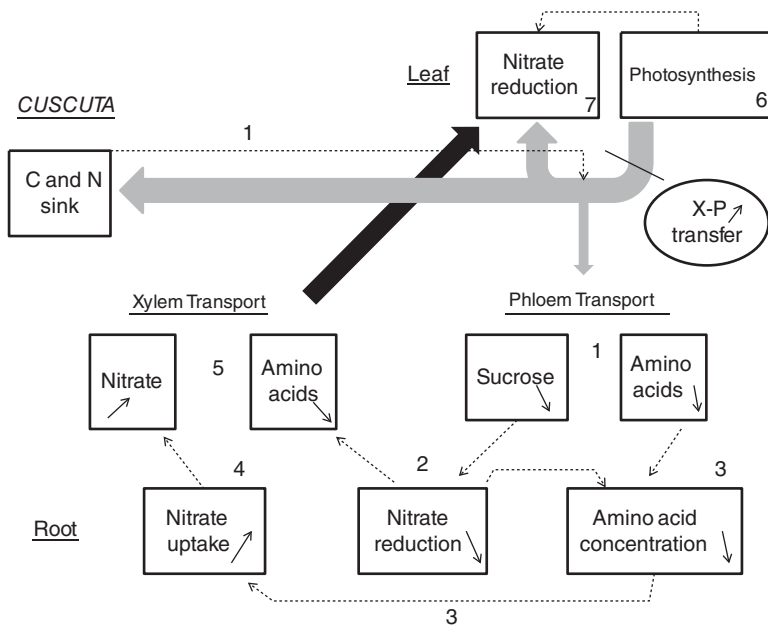


Fig. 7.13 Schematic representation of the interactions between *Cuscuta reflexa*, the flows of carbon (C) and nitrogen (N) within the host *Ricinus communis* and some basic facets of the C and N economy of the nitrate-fed host. The broad black arrow represents xylem transport, and the broad dark grey arrows represent phloem transport, of both C and N; dashed arrows denote effects or regulations; oblique black arrows indicate increases or decreases in reactions or concentrations. Jeschke & Hilpert 1997. Reproduced with permission of John Wiley & Sons.

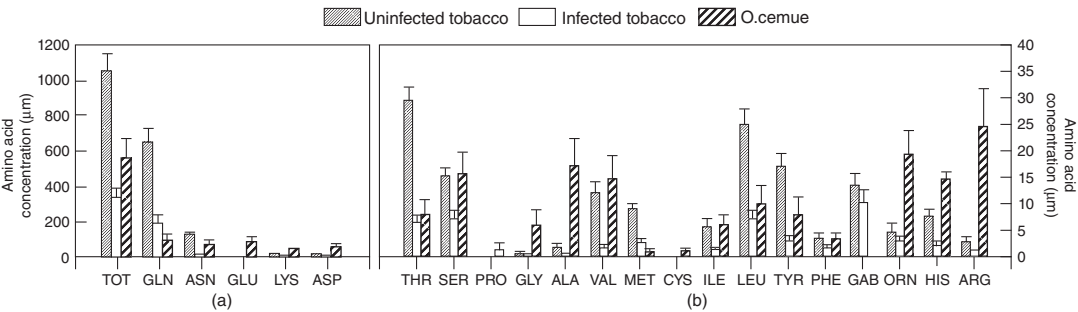


Fig. 7.14 The concentration (μM) of the total amino acid pool and individual amino acids in the xylem sap of the parasitic plant *O. cernua* and also its infected tobacco host and uninfected controls. (a) The concentration of total amino acids and abundant individual amino acids. (b) The concentration of less abundant amino acids. TOT, total amino acid pool; GLN, glutamine; ASN, asparagine; GAB, Gaba; GLU, glutamic acid; LYS, lysine; ASP, aspartate; THR, threonine; SER, serine; PRO, proline; GLY, glycine; ALA, alanine; VAL, valine; MET, methionine; CYS, cysteine; ILE, isoleucine; LEU, leucine; TYR, tyrosine; PHE, phenylalanine; ORN, ornithine; HIS, histidine; ARG, arginine. Hibberd *et al.* 1999. Reproduced with permission of John Wiley & Sons.

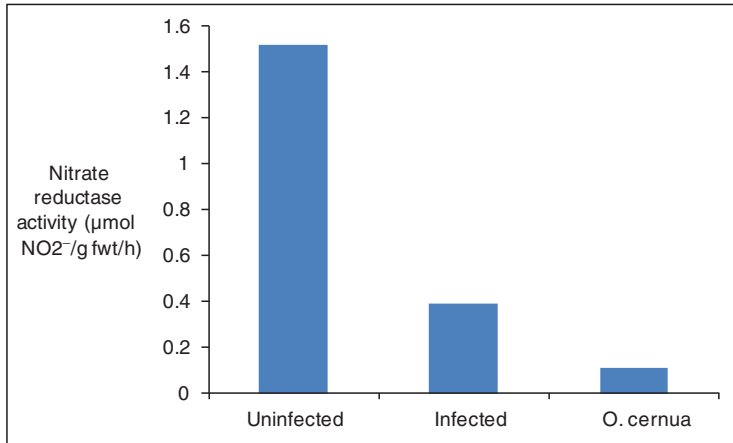


Fig. 7.15 Nitrate reductase activity in roots of uninfected tobacco plants, roots of plants infected with *Orobanche cernua* and in the parasite itself. Data from Hibberd *et al.* 1999.

the concentrations of glutamine, GABA and methionine in the xylem sap were lower in the parasite than in infected plants (Fig. 7.14). Interestingly, the activity of nitrate reductase in roots of infected plants was significantly lower than in uninfected plants and was even lower in the parasite itself (Fig. 7.15). The authors suggested that the lower nitrate reductase activity in roots of infected tobacco was due to a reduced supply of carbon to the roots, which down-regulated its activity, as well as the expression and synthesis of nitrate reductase protein (Glaab & Kaiser, 1993; Vincentz *et al.*, 1993).

Ola x phyllanthi is a xylem-tapping root hemiparasite of a wide range of host species in South West Australia. In this interaction, there are no open xylem-to-xylem connections, and nutrient abstraction occurs through the symplast via interfacial parenchyma (Pate *et al.*, 1990). On a favoured host, the nitrogen-fixing *Acacia littorea*, its effects on photosynthesis and nitrogen fixation are relatively small (Tennakoon *et al.*, 1997). However, it exerted substantial effects on partitioning of dry matter and nitrogen between root and shoot of the host. On the basis of the results obtained in their experiments, Tennakoon *et al.* (1997) constructed models comparing the flow and incorporation of nitrogen into dry matter in un-parasitised and parasitised plants (Fig. 7.16). These models show that by tapping the host's xylem stream, the parasite attracts more than 50% of the fixed nitrogen exported from the root nodules of the *Acacia*. The fixed nitrogen not diverted by the parasite moves first to the shoot in the xylem and is then cycled back to the infected host root. Roots of plants infected with *O. phyllanthi* gain considerably more nitrogen than uninfected roots, while shoots of parasitized plants lose a small amount of nitrogen (Fig. 7.16). On the basis of these data, the authors conclude that by withdrawing nitrogen from the host xylem, the parasite induced nitrogen deficiency in the host shoot, resulting in preferential deployment of remaining nitrogen towards increasing root growth (Tennakoon *et al.*, 1997).

Rhinanthus minor is a facultative root hemiparasite that can infect a range of hosts, including barley. Similarly to other hemiparasites, it taps the host xylem, thereby obtaining minerals and some organic compounds from the host. Despite the fact that they do not abstract phloem-borne assimilates from the host, they can still cause serious damage and

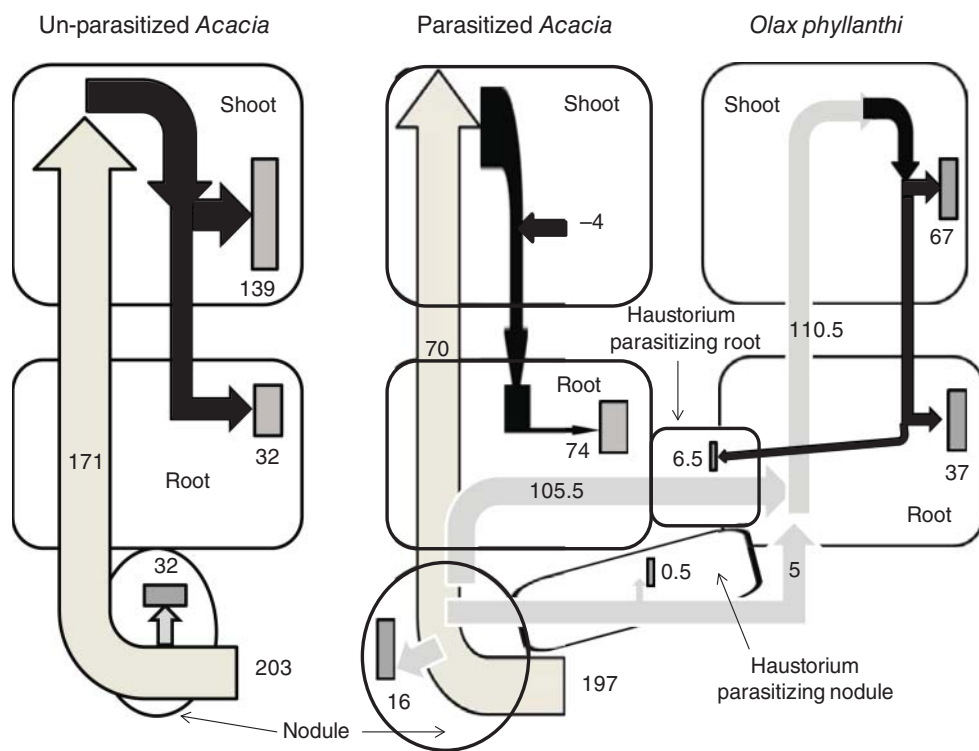


Fig. 7.16 Empirical model of net nitrogen flow in the xylem (white block arrows) and phloem (black block arrows) during a 4-month study period in unparasitized *Acacia littorea* and in *A. littorea* in the presence of the root hemiparasite *Olax phyllanthi*. The modelling procedure assumed that all net increments of nitrogen in both the host plant and the parasite came from nitrogen fixation of nodules of the legume host. The width of arrows (nitrogen flow via xylem and phloem) and the areas of squares (nitrogen increments in dry matter) are drawn in proportion to rates of flows, increments or losses. Tennakoon *et al.* 1997. Reproduced with permission of Oxford University Press.

growth reductions in their hosts (Chapter 2). On barley, *R. minor* was found to withdraw substantial amounts of xylem-borne nutrients via the open xylem-to-xylem connections (Jiang *et al.*, 2004). Compared to unattached *R. minor*, total nitrogen uptake was 15-fold greater in the parasite attached to its barley host (Fig. 7.17). This meant that the parasite was diverting 18% of the host's nitrogen uptake, which was already reduced by 18% after infection. This diversion of nitrogen in the xylem to the parasite meant that less nitrogen was available in the xylem flow to the host shoot (34% reduction compared to the unparasitised plant; Fig. 7.17). Nitrogen was not the only nutrient affected. We have already seen that by attaching to its barley host, the parasite was able to take up 15 times more nitrogen than in its unattached state. For phosphorus, uptake by the attached *R. minor* was 25 times greater than for the unattached parasite, while for potassium and magnesium, uptake was 19-fold and sevenfold greater, respectively (Jiang *et al.*, 2004).

In the interactions described previously, nitrogen extraction was greatest when the parasite, irrespective of whether it was a holoparasite (*C. reflexa*) or a hemiparasite (*O. phyllanthi*),

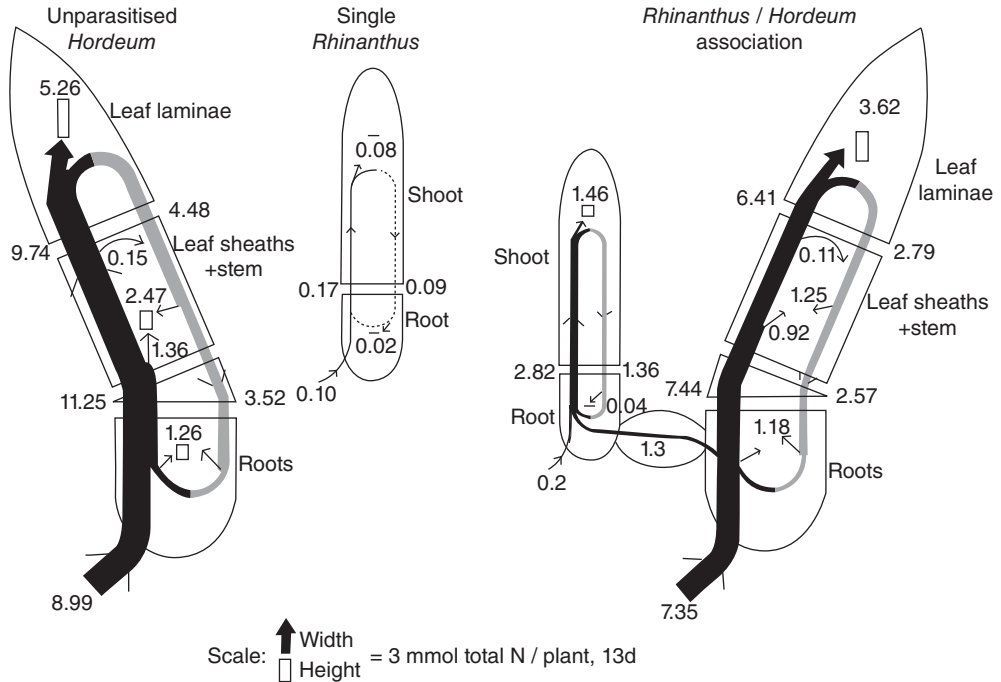


Fig. 7.17 Empirical models of the uptake of nitrate and the transport and utilisation of total nitrogen in whole plants of *Hordeum vulgare*, of solitary *Rhinanthus minor* and within the association of *Rhinanthus* parasitising *Hordeum* over the 13-day study period starting 41 day after sowing, approximately 30 day after attachment of the parasite. Numbers are presented in mmol N per plant over 13 day. The width of arrows [net flows in xylem (black) or phloem (dotted)] and the height of histograms (deposition of total N in each organ) are drawn in proportion to the rates of flows or to the magnitude of deposition (see the scale). The triangle between root and leaf sheaths indicates the stem. Jiang *et al.* 2004. Reproduced with permission of CSIRO Publishing.

was attached to a nitrogen-fixing leguminous host. The figures for extraction of nitrogen from the host are 214% and 56% of current nitrogen fixation for *C. reflexa* and *O. phyllanthi*, respectively. On non-nitrogen-fixing hosts, nitrogen extraction is considerably less, 28% for the holoparasite *O. cernua* on tobacco and 18% for the hemiparasite *R. minor* on barley. As Jiang *et al.* (2004) point out, the smaller nitrogen extraction by the hemiparasites can be related to their more moderate effects on host growth compared to the holoparasites. However, not all hemiparasites exert moderate effects on growth of their hosts. As we saw in Chapter 2, *Striga* can exert very substantial and highly damaging effects on host growth.

7.6 CONCLUSIONS

Attack on plants by pathogens, nematodes, insects and parasitic plants can result in large perturbations in plant nutrient relations. Although some of these alterations can be explained, in part, by changes in water uptake and transport resulting from attack (Chapter 6), more often,

the mechanisms underlying changes in nutrient relations are more complex. Indeed, as we have seen previously, some of the changes, for example in nitrogen, are the result of reallocation by the plant away from sites of attack. The more we learn about the responses of plants to attack, the more we become aware of the dynamic nature of plant interactions with the biotic environment. Even in susceptible interactions, where attackers can seemingly overrun plants, the host is capable of adjusting its physiological responses in an attempt to minimise damage. Plant and attacker are engaged in an intimate struggle for metabolic supremacy (or survival), and as we will see in the next chapter (Chapter 8), plant hormones can be pivotal in the final outcome of this struggle.

RECOMMENDED READING

- Hibberd JM, Jeschke WD, 2001a. Solute flux into parasitic plants. *Journal of Experimental Botany* **52**, 2043–2049.
- Newingham BA, Callaway RM, BassiriRad H, 2007a. Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. *Oecologia* **153**, 913–920.
- Tao L, Hunter MD, 2013a. Allocation of resources away from sites of herbivory under simultaneous attack by aboveground and belowground herbivores in the common milkweed, *Asclepias syriaca*. *Arthropod-Plant Interactions* **7**, 217–224.

REFERENCES

- AbuQamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, Lam S, Dietrich RA, Mengiste T, 2006. Expression profiling and mutant analysis reveals complex regulatory networks involved in *Arabidopsis* response to *Botrytis* infection. *The Plant Journal* **48**, 28–44.
- Ahmad I, Ower SAP, Farrar JF, Whitbread R, 1982. The distribution of five major nutrients in barley plants infected with brown rust. *Physiological Plant Pathology* **21**, 335–346.
- Asher MJC, 1972. Effect of *Ophiobolus graminis* infection on the assimilation and distribution of ^{14}C in wheat. *Annals of Applied Biology* **72**, 161–167.
- Ayres PG, Jones P, 1975. Increased transpiration and the accumulation of root absorbed ^{86}Rb in barley leaves infected by *Rhynchosporium secalis* (leaf blotch). *Physiological Plant Pathology* **7**, 49–58.
- Ayres E, Heath J, Possell M, Black HJ, Kerstiens G, Bardgett RD, 2004. Tree physiological responses to above-ground herbivory directly modify below-ground processes of soil carbon and nitrogen cycling. *Ecology Letters* **7**, 469–479.
- Ayres E, Dromph KM, Cook R, Ostle N, Bardgett RD, 2007. The influence of below-ground herbivory and defoliation of a legume on nitrogen transfer to neighbouring plants. *Functional Ecology* **21**, 256–263.
- Babst BA, Ferrieri RA, Gray DW, Lerda M, Schlyer DJ, Schueller M, Thorpe MR, Orians CM, 2005. Jasmonic acid induces rapid changes in carbon transport and partitioning in *Populus*. *New Phytologist* **167**, 63–72.
- Baldwin JG, Barker KR, Nelson LA, 1979. Effect of *Meloidogyne incognita* on nitrogen fixation in soybean. *Journal of Nematology* **11**, 156–161.
- Barker KR, Hussey RS, 1976. Histopathology of nodular tissues of legumes infected with certain nematodes. *Phytopathology* **66**, 851–855.
- Bäumel P, Jeschke WD, Witte L, Czygan F-C, Proksch P, 1993. Uptake and transport of quinolizidine alkaloids in *Cuscuta reflexa* parasitizing on *Lupinus angustifolius*. *Zeitschrift für Naturforschung* **48c**, 436–443.
- Baxter L, Tripathy S, Ishaque N, Boot N, Cabral A, *et al.*, 2010. Signatures of adaptation to obligate biotrophy in the *Hyaloperonospora arabidopsidis* genome. *Science* **330**, 1549–1551.
- Brauc S, De Vooght E, Claeys M, Hofte M, Angenon G, 2011. Influence of over-expression of cytosolic aspartate aminotransferase on amino acid metabolism and defence responses against *Botrytis cinerea* infection in *Arabidopsis thaliana*. *Journal of Plant Physiology* **168**, 1813–1819.
- Brauc S, De Vooght E, Claeys M, Geuns JMC, Hofte M, Angenon G, 2012. Overexpression of arginase in *Arabidopsis thaliana* influences defence responses against *Botrytis cinerea*. *Plant Biology* **14**, 39–45.

- Clarkson DT, Drew MC, Ferguson IB, Sanderson J, 1975. The effect of the take-all fungus, *Gaeumannomyces graminis*, on the transport of ions by wheat plants. *Physiological Plant Pathology* **6**, 75–84.
- Daroub SH, Snyder GH, 2007. The chemistry of plant nutrients in soil. In: Datnoff LE, Elmer WH, Huber DM, eds. *Mineral nutrition and plant disease*. St. Paul, MN: APS Press, pp. 1–7.
- De Ruijter FJ, Haverkort AJ, 1999. Effects of potato cyst nematodes (*Globodera pallida*) and soil pH on root growth, nutrient uptake and crop growth of potato. *European Journal of Plant Pathology* **105**, 61–76.
- Divon HH, Ziv C, Davydov O, Yarden O, Fluhr R, 2006. The global nitrogen regulator, FNR1, regulates fungal nutrition genes and fitness during *Fusarium oxysporum* pathogenesis. *Molecular Plant Pathology* **7**, 485–497.
- Dörr I, 1972. Der Anschluß der Cuscuta-Hyphen an die Siebröhren ihrer Wirtspflanzen. *Protoplasma* **75**, 167–184.
- Duplessis S, Hacquard S, Delaruelle C, Tisserant E, Frey P, *et al.*, 2011. *Melampsora larici-populina* transcript profiling during germination and time-course infection of poplar leaves reveals dynamic expression patterns associated with virulence and biotrophy. *Molecular Plant-Microbe Interactions* **24**, 808–818.
- Evans K, 1982. Effects of infestation with *Globodera pallida* (Wollenweber) Behrens Ro1 on the growth of four potato cultivars. *Crop Protection* **1**, 169–179.
- Evans K, Franco J, 1979. Tolerance to cyst-nematode attack in commercial potato cultivars and some possible mechanisms for its operation. *Nematologica* **25**, 153–162.
- Fitt BDL, Hornby D, 1978. Effects of root-infecting fungi on wheat transport processes and growth. *Physiological Plant Pathology* **13**, 335–346.
- Fric F, 1975. Translocation of ¹⁴C-labelled assimilates in barley plants infected with powdery mildew. *Phytopathologische Zeitschrift* **84**, 88–95.
- Frost CJ, Hunter MD, 2008. Herbivore-induced shifts in carbon and nitrogen allocation in red oak seedlings. *New Phytologist* **178**, 835–845.
- Gerwitz DL, Durbin RD, 1965. The influence of rust on the distribution of P³² in the bean plant. *Phytopathology* **55**, 57–61.
- Gheysen G, Fenoll C. 2002. Gene expression in nematode feeding sites. *Annual Review of Phytopathology* **40**, 191–219.
- Givan CV, 1979. Metabolic detoxification of ammonia in tissues of higher plants. *Phytochemistry* **18**, 375–382.
- Glaab J, Kaiser WM, 1993. Rapid modulation of nitrate reductase in pea roots. *Planta* **191**, 173–179.
- Gómez S, Ferrieri RA, Schueller M, Orians CM, 2010. Methyl jasmonate elicits rapid changes in carbon and nitrogen dynamics in tomato. *New Phytologist* **188**, 835–844.
- Grundler FMW, Betka M, Wyss U, 1991. Influence of changes in the nurse cell system (syncytium) on sex determination and development of the cyst nematode *Heterodera schachtii*: total amounts of proteins and amino acids. *Phytopathology* **81**, 70–74.
- Hamilton EWI, Frank DA, 2001. Can plants stimulate microbes and their own nutrient supply? Evidence from a grazing tolerant grass. *Ecology* **82**, 2397–2404.
- Hestbjerg H, Fielding G, Elmholt S, 2002. *Fusarium culmorum* infection of barley seedlings: correlation between aggressiveness and deoxynivalenol content. *Journal of Phytopathology* **150**, 308–312.
- Hibberd JM, Jeschke WD, 2001b. Solute flux into parasitic plants. *Journal of Experimental Botany* **52**, 2043–2049.
- Hibberd JM, Quick WP, Press MC, Scholes JD, Jeschke WD, 1999. Solute fluxes from tobacco to the parasitic angiosperm *Orobancha cernua* and the influence of infection on host carbon and nitrogen relations. *Plant, Cell and Environment* **22**, 937–947.
- Hofmann J, Wiczorek K, Blöchl A, Grundler FMW, 2007. Sucrose supply to nematode-induced syncytia depends on the apoplastic and the symplastic pathway. *Journal of Experimental Botany* **58**, 1591–1601.
- Hornby D, Fitt BDL, 1981. Effects of root-infecting fungi on structure and function of cereal roots. In: Ayres PG, ed. *Effects of disease on the physiology of the growing plant*. Cambridge, UK: Cambridge University Press, pp. 101–130.
- Horst RJ, Doehlemann G, Wahl R, Hofmann J, Schmiedl A, Kahmann R, Kämper J, Sonnewald U, Voll LM, 2010. *Ustilago maydis* infection strongly alters organic nitrogen allocation in maize and stimulates productivity of systemic source leaves. *Plant Physiology* **152**, 293–308.
- Hwang S, An SH, Hwang BK, 2011. Pepper *asparagine synthetase 1* (*CaASI*) is required for plant nitrogen assimilation and defense responses to microbial pathogens. *The Plant Journal* **67**, 749–762.

- Ibewiro B, Sangina N, Vanlauwe B, Merckx R, 2000. Influence of phytoparasitic nematodes on symbiotic N₂ fixation in tropical herbaceous legume cover crops. *Biology and Fertility of Soils* **31**, 254–260.
- Jammes F, Lecomte P, de Almeida-Engler J, Bitton F, Martin-Magniette M-L, Renou JP, Abad P, Favery B. 2005. Genome-wide expression profiling of the host response to root-knot nematode infection in *Arabidopsis*. *The Plant Journal* **44**, 447–458.
- Jeschke WD, Hilpert A, 1997. Sink-stimulated photosynthesis and sink-dependent increase in nitrate uptake: nitrogen and carbon relations of the parasitic association *Cuscuta reflexa* – *Ricinus communis*. *Plant, Cell and Environment* **20**, 47–56.
- Jeschke WD, Bäumel P, R  th N, Czygan F-C, Proksch P, 1994. Modelling of the flows and partitioning of carbon and nitrogen in the holoparasite *Cuscuta reflexa* Roxb. and its host *Lupinus albus* L. II. Flows between host and parasite and within the parasitized host. *Journal of Experimental Botany* **45**, 801–812.
- Jiang F, Jeschke WD, Hartung W, 2004. Solute flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite nutrient relations. *Functional Plant Biology* **31**, 633–643: <http://www.publish.csiro.au/nid/102/paper/FP03225.htm>
- Jones MGK, Northcote DH. 1972. Nematode-induced syncytium – a multinucleate transfer cell. *Journal of Cell Science* **10**, 789–809.
- Kang S, Kim H, Lee H, Choi J, Heu S, Oh C, Kwon S, An C, 2006. Overexpression in *Arabidopsis* of a plasma membrane-targeting glutamate receptor from small radish increases glutamate-mediated Ca²⁺ influx and delays fungal infection. *Molecules and Cells* **21**, 418–427.
- Lau GW, Hamer JE, 1996. Regulatory genes controlling *mgp1* expression and pathogenicity in the rice blast fungus *Magnaporthe grisea*. *The Plant Cell* **8**, 771–781.
- Lea PJ, Fowden L, 1975. The purification and properties of glutamine dependent asparagine synthetase isolated from *Lupinus albus*. *Proceedings of the Royal Society of London, B* **192**, 13–26.
- Liu GS, Ji YY, Bhuiyan NH, Pilot G, Selvaraj G, Zou JT, Wei YD. 2010. Amino acid homeostasis modulates salicylic acid-associated redox status and defense responses in *Arabidopsis*. *Plant Cell* **22**, 3845–3863.
- Macdonald AJ, Gutteridge RJ, 2012. Effect of take-all (*Gaeumannomyces graminis* var. *tritici*) on crop N uptake and residual mineral N in soil at harvest of winter wheat. *Plant and Soil* **350**, 253–260.
- Masclaux C, Valadier M, Brugi  re N, Morot-Gaudry J, Hirel B. 2000. Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. *Planta* **211**, 510–518.
- Maurel C, Chrispeels MJ. 2001. Aquaporins. A molecular entry into plant water relations. *Plant Physiology* **125**, 135–138.
- Mengel K, Viro M, 1978. The significance of plant energy status for the uptake and incorporation of NH₄⁺ nitrogen by young rice plants. *Soil Science and Plant Nutrition* **24**, 407–416.
- Murray AJS, Ayres PG, 1986. Uptake and translocation of nitrogen by mildewed barley seedlings. *New Phytologist* **104**, 355–365.
- Newingham BA, Callaway RM, BassiriRad H, 2007b. Allocating nitrogen away from a herbivore: a novel compensatory response to root herbivory. *Oecologia* **153**, 913–920.
- Olea F, P  rez-Garc  a A, Canton FR, Rivera EM, Canas R, Avila C, Cazorla FM, Canovas FM, De Vicente A, 2004. Up-regulation and localization of asparagine synthetase in tomato leaves infected by the bacterial pathogen *Pseudomonas syringae*. *Plant Cell Physiology* **45**, 770–780.
- Pageau K, Reisdorf-Cren M, Morot-Gaudry J-F, Masclaux-Daubresse C, 2006. The two senescence-related markers *GSI* (cytosolic glutamine synthetase) and *GDH* (glutamate dehydrogenase), involved in nitrogen mobilisation are differentially regulated during pathogen attack, by stress hormones and reactive oxygen species in *Nicotiana tabacum* L. *Journal of Experimental Botany* **57**, 547–557.
- Pate JS, Kuo J, Davidson NJ, 1990. Morphology and anatomy of the haustorium of the root hemiparasite *Oxalophyllanthi* (Oxalaceae), with special reference to the haustorial interface. *Annals of Botany* **65**, 425–436.
- P  rez-Garc  a A, Canovas FM, Gallardo F, Hirel B, De Vicente A, 1995. Differential expression of glutamine synthetase isoforms in tomato detached leaflets infected with *Pseudomonas syringae* pv. *tomato*. *Molecular Plant-Microbe Interactions* **8**, 96–103.
- P  rez-Garc  a A, Pereira S, Pissara J, Garc  a Guti  rrez FM, Cazorla FM, Salema R, De Vicente A, Canovas FM, 1998. Cytosolic localization in tomato mesophyll cells of a novel glutamine synthetase induced in response to bacterial infection or phosphinothricin treatment. *Planta* **206**, 426–434.
- P  rez-Garc  a A, Pereira S, Pissarra J, Guti  rrez AG, Cazorla FM, Salema R, de Vicente A, Canovas FM, 1998a. Cytosolic localization in tomato mesophyll cells of a novel glutamine synthetase induced in response to bacterial infection or phosphinothricin treatment. *Planta* **206**, 426–434.

- Perez-Garcia A, de Vicente A, Canton FR, Cazorla FM, Codina JC, Garcia-Gutierrez A, Canovas FM, 1998b. Light-dependent changes of tomato glutamine synthetase in response to *Pseudomonas syringae* infection or phosphinothricin treatment. *Physiologia Plantarum* **102**, 377–384.
- Petre B, Morin E, Tisserant E, Hacquard S, Da Silva C, Poulain J, Delaruelle C, Martin F, Rouhier N, Kohler A, Duplessis S, 2012. RNA-seq of early-infected poplar leaves by the rust pathogen *Melampsora larici-populina* uncovers *PtSultr3;5*, a fungal-induced host sulfate transporter. *PLOS One* **7**(8), e44408.
- Piening LJ, 1972. Effects of leaf rust on nitrate in rye. *Canadian Journal of Plant Science* **52**, 842–843.
- Rice RW, 2007. The physiological role of minerals in the plant. In: Datnoff LE, Elmer WH, Huber DM, eds. *Mineral nutrition and plant disease*. St. Paul, MN: APS Press, pp. 9–29.
- Roberts AM, Walters DR, 1988. Nitrogen assimilation and metabolism in rusted leek leaves. *Physiological and Molecular Plant Pathology* **32**, 229–235.
- Rohringer R, 1957. Untersuchungen zur Biochemie von weizen Keimpflanzen nach Infektion mit *Puccinia graminis tritici*. *Phytopathologische Zeitschrift* **29**, 45–64.
- Sadler R, Scott KJ, 1974. Nitrogen assimilation and metabolism in barley leaves infected with the powdery mildew fungus. *Physiological Plant Pathology* **4**, 235–247.
- Schoeny A, Devienne-Barret F, Jeuffroy M-H, Lucas P, 2003. Effect of take-all root infections on nitrate uptake in winter wheat. *Plant Pathology* **52**, 52–59.
- Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT, 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. *Proceedings of the National Academy of Sciences USA* **103**, 12935–12940.
- Seifi HS, Van Brockhaven J, Angenon G, Hofte M, 2013a. Glutamate metabolism in plant disease and defense: friend or foe? *Molecular Plant-Microbe Interactions* **26**, 475–485.
- Seifi HS, Curvers K, De Vleeschauwer D, Delaere I, Aziz A, Hofte M, 2013b. Concurrent overactivation of the cytosolic glutamine synthetase and the GABA shunt in the ABA-deficient sitiens mutant of tomato leads to resistance against *Botrytis cinerea*. *New Phytologist* **199**, 490–504.
- Sijmons PC, Grundler FMW, Von Mende N, Burrows PR, Wyss U, 1991. *Arabidopsis thaliana* as a new model host for plant parasitic nematodes. *Plant Journal* **1**, 245–254.
- Smith AM, Coupland G, Dolan L, Harberd N, Jones J, Martin C, Sablowski R, Amey A, 2010. *Plant biology*. New York: Garland Science.
- Snoeijers SS, Pérez-Garcia A, Joosten MHAI, De Wit PJGM, 2000. The effect of nitrogen on disease development and gene expression in bacterial and fungal pathogens. *European Journal of Plant Pathology* **106**, 493–506.
- Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, *et al.*, 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**, 1543–1546.
- Swartzberg D, Kirshner B, Rav-David D, Elad Y, Granot D, 2008. *Botrytis cinerea* induces senescence and is inhibited by autoregulated expression of the IPT gene. *European Journal of Plant Pathology* **120**, 289–297.
- Tao L, Hunter MD, 2011. Effects of insect herbivores on the nitrogen economy of plants. In: Polacco JC, Todd CD, eds. *Ecological aspects of nitrogen metabolism in plants*. West Sussex, UK: Wiley-Blackwell, pp. 255–279.
- Tao L, Hunter MD, 2013b. Allocation of resources away from sites of herbivory under simultaneous attack by aboveground and belowground herbivores in the common milkweed, *Asclepias syriaca*. *Arthropod-Plant Interactions* **7**, 217–224.
- Tavernier V, Cadiou S, Pageau K, Laugé R, Reisdorf-Cren M, Langin T, Masclaux-Daubresse C, 2007. The plant nitrogen mobilization promoted by *Colletotrichum lindemuthianum* in *Phaseolus* leaves depends on fungus pathogenicity. *Journal of Experimental Botany* **58**, 3351–3360.
- Tennakoon KU, Pate JS, Fineran BA, 1997. Growth and partitioning of C and fixed N in the shrub legume *Acacia littorea* in the presence or absence of the root hemiparasite *Oxalophyllanthi*. *Journal of Experimental Botany* **48**, 1047–1060.
- Thomma BPHJ, Bolton MD, Clergeot PH, De Wit PJGM, 2006. Nitrogen controls *in planta* expression of *Cladosporium fulvum* Avr9 but no other effector genes. *Molecular Plant Pathology* **7**, 125–130.
- Trudgill DL, Cotes LM, 1983. Tolerance of potato to potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) in relation to the growth and efficiency of the root system. *Annals of Applied Biology* **102**, 385–397.
- Trudgill DL, Evans K, Parrott DM, 1975a. Effects of potato cyst nematodes on potato plants. I. Effects in a trial with irrigation and fumigation on the growth and nitrogen and potassium contents of a resistant and a susceptible variety. *Nematologica* **21**, 169–182.

- Trudgill DL, Evans K, Parrott DM, 1975b. Effects of potato cyst nematodes on potato plants. II. Effects of haulm size, concentration of nutrients in haulm tissue and tuber yield of a nematode resistant and a nematode susceptible variety. *Nematologica* **21**, 183–191.
- Van Baarlen P, Staats M, Van Kan JAL, 2004. Induction of programmed cell death in lily by the fungal pathogen *Botrytis elliptica*. *Molecular Plant Pathology* **5**, 559–574.
- Van Doorn WG, Beers EP, Dangl JL, Franklin-Tong VE, Gallois P, Hara-Nishimura I, Jones AM, Kawai-Yamada M, Lam E, Mundy J, Mur LAJ, Petersen M, Smertenko A, Taliansky M, Van Breusegem F, Wolpert T, Woltering E, Zhivotovsky B, Bozhkov PV, 2011. Morphological classification of plant cell deaths. *Cell Death and Differentiation* **18**, 1241–1246.
- Vincenz M, Moureaux T, Legdecker MT, Vaucheret T, Caboche M, 1993. Regulation of nitrate and nitrite reductase in *Nicotiana plumbaginifolia* leaves by nitrogen and carbon metabolism. *The Plant Journal* **3**, 1027–1035.
- Walters DR, 1981. Root function in mildewed barley. *PhD thesis, Lancaster University*.
- Walters DR, 1985. Shoot: root interrelationships: the effects of obligately biotrophic fungal pathogens. *Biological Reviews* **60**, 47–79.
- Walters DR, 2010. *Plant defense. Warding off attack by pathogens, herbivores and parasitic plants*. Oxford: Wiley-Blackwell.
- Walters DR, Ayres PG, 1980. Effects of powdery mildew disease on uptake and metabolism of nitrogen by roots of infected barley. *Physiological Plant Pathology* **17**, 369–379.
- Walters DR, Ayres PG, 1981. ³²P uptake and transport by roots of powdery mildew infected barley. *Physiological Plant Pathology* **18**, 195–205.
- Walters DR, Ayres PG, 1982. Water movement through root systems excised from healthy and mildewed barley: relationships with phosphate transport. *Physiological Plant Pathology* **20**, 275–284.
- Wu H-S, Yin X-M, Zhu Y-Y, Guo S-W, Wu C-L, Lu Y-L, Shen Q-R, 2007. Nitrogen metabolism disorder in watermelon leaf caused by fusaric acid. *Physiological and Molecular Plant Pathology* **71**, 69–77.

8 Hormonal Changes in Plants Under Attack

8.1 INTRODUCTION

Plant hormones are important in regulating plant responses to a wide range of biotic stresses. These hormones include abscisic acid (ABA), auxin, gibberellic acid (GA), cytokinin (CK) and ethylene (ET), as well as salicylic acid (SA), jasmonic acid (JA), brassinosteroids (BRs) and the strigolactones, identified in 2008 as a new class of plant hormones (Gomez-Roldan *et al.*, 2008; Umehara *et al.*, 2008). Infection of plants with pathogens or parasitic plants, as well as attack by nematodes or insect herbivores, can lead to changes in the concentrations or distribution of hormones. Some of these hormonal changes are associated with altered plant growth and development, including various growth abnormalities. However, in recent years, experimental evidence has highlighted important roles for hormones in plant defence. These are exciting times for those studying plant interactions with the biotic environment, as new and powerful experimental tools allow researchers to unravel the complexity of plant interactions to attackers.

In this chapter we examine how plant hormones are affected by interaction with various types of attacker. The main focus is on plant responses and the consequences, to both the plants and their attacker, of changes in hormone levels and signalling. Details of the modes of action of the various plant hormones are beyond the scope of this chapter, and readers are referred to the clear explanations of hormone modes of action provided by Hodson and Bryant (2012).

8.2 HORMONAL CHANGES IN PLANTS RESPONDING TO PATHOGENS

Plant infection by certain pathogens leads to abnormal growth, including the formation of galls and tumours, excessive branching and the abnormal induction of adventitious roots. Such symptoms are consistent with changes in the hormonal status of the plant, although trying to determine the role played by hormones in the development of such symptoms has proved difficult, partly because changes in more than one hormone might be involved and also because

some pathogens are known to produce hormones. However, it would be wrong to think that hormones are only involved in abnormal growth development in diseased plants. Indeed, we now know that plant hormones are important players in various aspects of the plant–pathogen interaction, including defence. With this in mind, let us start our journey through the hormonal changes that occur in diseased plants by looking briefly at SA, JA and ET. Readers interested in a more detailed discussion of the roles of SA, JA and ET in plant defence should refer to the excellent recent review by Pieterse *et al.* (2012).

8.2.1 Salicylic acid, jasmonic acid and ethylene

These three hormones play important roles in regulating plant defences against attack (Walters, 2010; Pieterse *et al.*, 2012a, b). Thus, SA plays a crucial role in plant defence and is involved in activating defences against biotrophic and hemibiotrophic pathogens. Mutants that are unable to accumulate SA, or are insensitive to SA, exhibit enhanced susceptibility to such pathogens. In contrast, JA and ET are associated with defence against necrotrophic pathogens and chewing insects. For example, JA signalling is activated in response to attack by *Manduca sexta* caterpillars in tobacco and caterpillars of *Pieris rapae* as well as thrips in *Arabidopsis* (Reymond *et al.*, 2004; De Vos *et al.*, 2005). Interestingly, however, not all insect herbivores activate JA signalling. The silverleaf whitefly *Bemisia tabaci* activates SA signalling and suppresses JA signalling in *Arabidopsis* (Kempema *et al.*, 2007), suggesting that defence against sucking/piercing insects might be mediated by SA rather than JA.

The SA-mediated and JA/ET-mediated defence pathways tend to be mutually antagonistic, although there are reports of synergistic interactions (Pieterse *et al.*, 2012a, b). Increasing evidence suggests that the defence signalling pathway activated in an attacked plant depends on pathogen lifestyle, with positive or negative crosstalk between the SA and JA/ET pathways regulated to ensure an appropriate and effective defence response. What has become clear in recent years is that the plant defence signalling mediated by SA, JA and ET can be modulated by other plant hormones, such as ABA, auxin, cytokinins and gibberellins.

8.2.2 Absciscic acid

8.2.2.1 ABA and vascular wilts

ABA is involved in regulating various aspects of plant growth and development, including seed germination, leaf senescence, stomatal aperture and adaption to environmental stresses (Wasilewskaa *et al.*, 2008). As we saw in Chapter 6, infection by vascular wilt pathogens can lead to wilting and stunting of plants. Xylem vessels become blocked due to the production of gums and mucilage, growth of fungal hyphae in the lumen of vessels and the production of tyloses, leading to impairment of water flow and, ultimately, to the development of water stress. When endogenous levels of ABA were examined in tomato isolines resistant and susceptible to the vascular wilt pathogen *Verticillium albo-atrum*, marked increases were found in young leaves and stems of susceptible plants compared to stems and leaves of resistant, symptomless plants (Fig. 8.1; Pegg, 1981). These increases in ABA levels in susceptible plants were thought to be the result of pathogen-induced water stress. One disease where it might be expected to find increased ABA levels is lethal yellowing of coconut. This disease, caused by phytoplasmas, leads to permanent stomatal closure, decreased photosynthesis, leaf yellowing and, ultimately, plant death. Because of the involvement of ABA in stomatal closure, Martinez *et al.* (2000) measured ABA levels in roots, leaves and xylem sap of coconut palms at

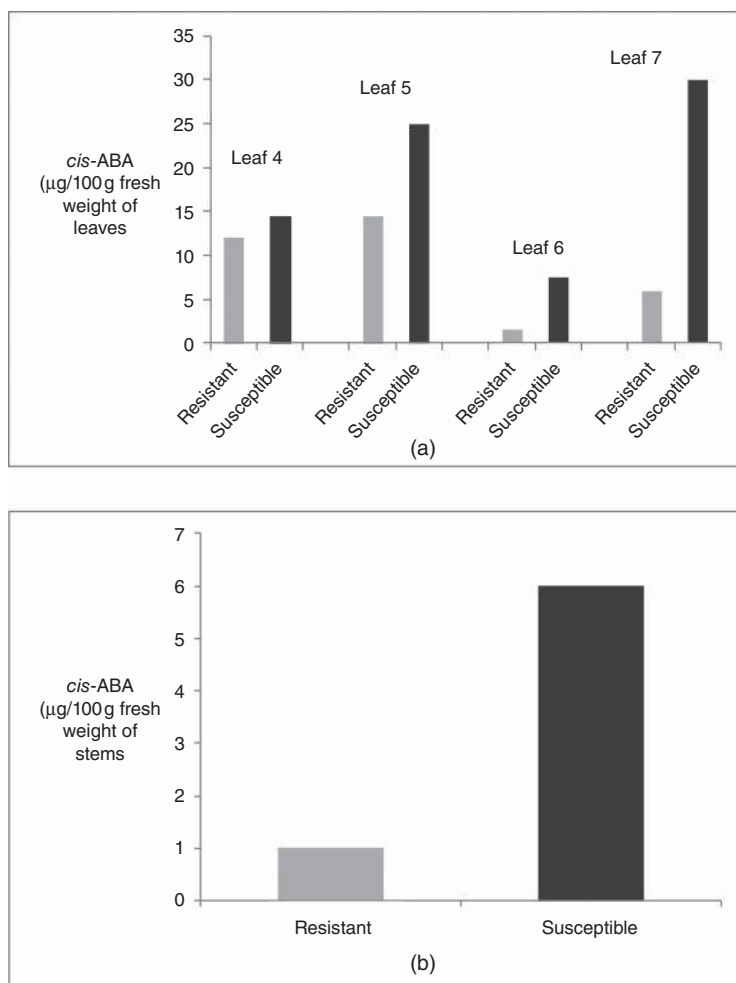


Fig. 8.1 Levels of *cis*-ABA in leaves (a) and stems (b) of resistant and susceptible tomato plants, cultivar Craigella, after inoculation with *Verticillium albo-atrum*. Pegg 1981. Reproduced with permission of Cambridge University Press.

different stages of disease development. They found that although ABA concentrations in leaf and xylem sap extracts increased with disease progression, these increases did not occur until the later stages of disease, well after abnormal stomatal closure (Fig. 8.2). Furthermore, ABA concentrations in roots were not correlated with stomatal closure. These results suggested that bulk ABA concentrations in leaf xylem sap and leaf tissue were not responsible for abnormal stomatal closure in lethal yellowing disease of coconut.

8.2.2.2 ABA and plant defence

Although it has long been known that ABA plays important roles in plant responses to abiotic stress, research in the past 10–15 years has provided evidence that ABA also

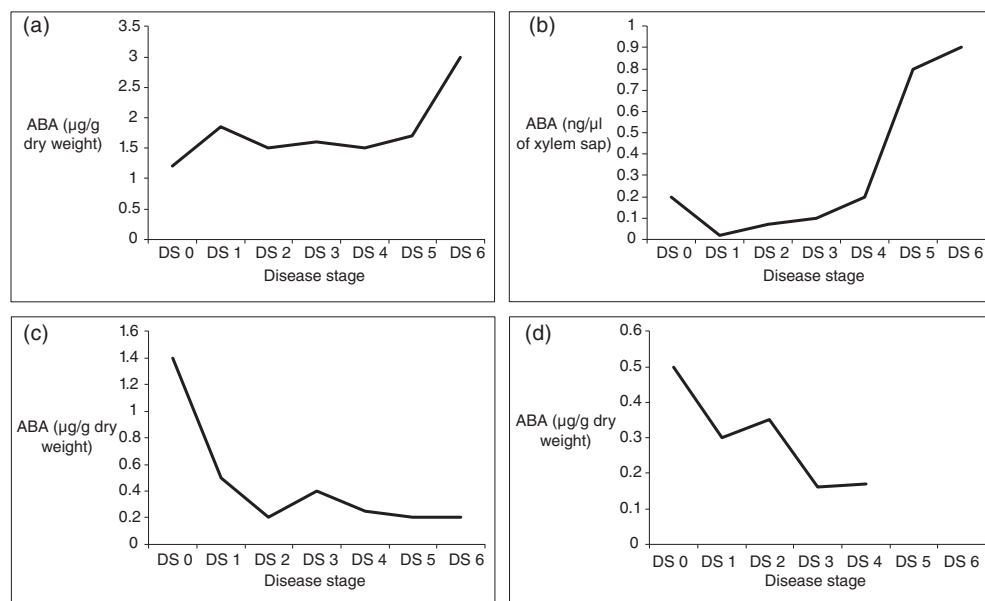


Fig. 8.2 ABA concentrations in (a) leaves, (b) xylem sap, (c) primary roots and (d) secondary roots of coconut palms with different stages of lethal yellowing disease. Stage 0 corresponds to symptomless palms. In stages 5 and 6, no secondary roots were found. Martinez *et al.* 2000. Reproduced with permission of Elsevier.

plays a prominent role in plant responses to biotic stress. In fact, ABA has emerged as an important regulator of biotic defence responses, promoting resistance in some plant–pathogen interactions but increasing susceptibility in others. For example, tomato plants deficient in ABA (*sitiens* mutants) were more resistant to various pathogens, including *Botrytis cinerea* (Audenaert *et al.*, 2002), *Oidium neolycopersici* (Achuo *et al.*, 2006) and *Erwinia chrysanthemi* (Asselbergh *et al.*, 2008) than wild-type plants. In a similar vein, *Arabidopsis* mutants impaired in their ability to synthesise ABA exhibited enhanced resistance to *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (de Torres-Zabala *et al.*, 2007). Bacterial pathogens such as *Pst* use an array of chemical virulence factors and proteinaceous effectors in their interaction with host plants. The effectors are delivered into plant cells via a type III protein secretion system (T3SS). These type III effectors (T3Es) appear to interfere with host signalling and metabolism, thereby promoting suppression of basal defence and enhancing pathogen nutrition. de Torres-Zabala *et al.* (2007) examined ABA levels in *Arabidopsis* challenged with the virulent *Pst* strain DC3000, as well as strain DC3000*hrpA*[−], which is a mutant compromised in T3SS. They found that ABA levels in the host increased rapidly and by 18 hours after inoculation were some 10-fold greater in plants challenged with DC3000 compared to mock-challenged plants and those challenged with DC3000*hrpA*[−] (Fig. 8.3a). These increases in ABA levels were associated with increased expression of the ABA biosynthetic gene *NCED3* following challenge with DC3000 (Fig. 8.3b). Interestingly, multiplication of the virulent *Pst* DC3000 in an *Arabidopsis* ABA biosynthetic mutant was significantly reduced compared to the wild type (Fig. 8.3c). These results show that bacterial

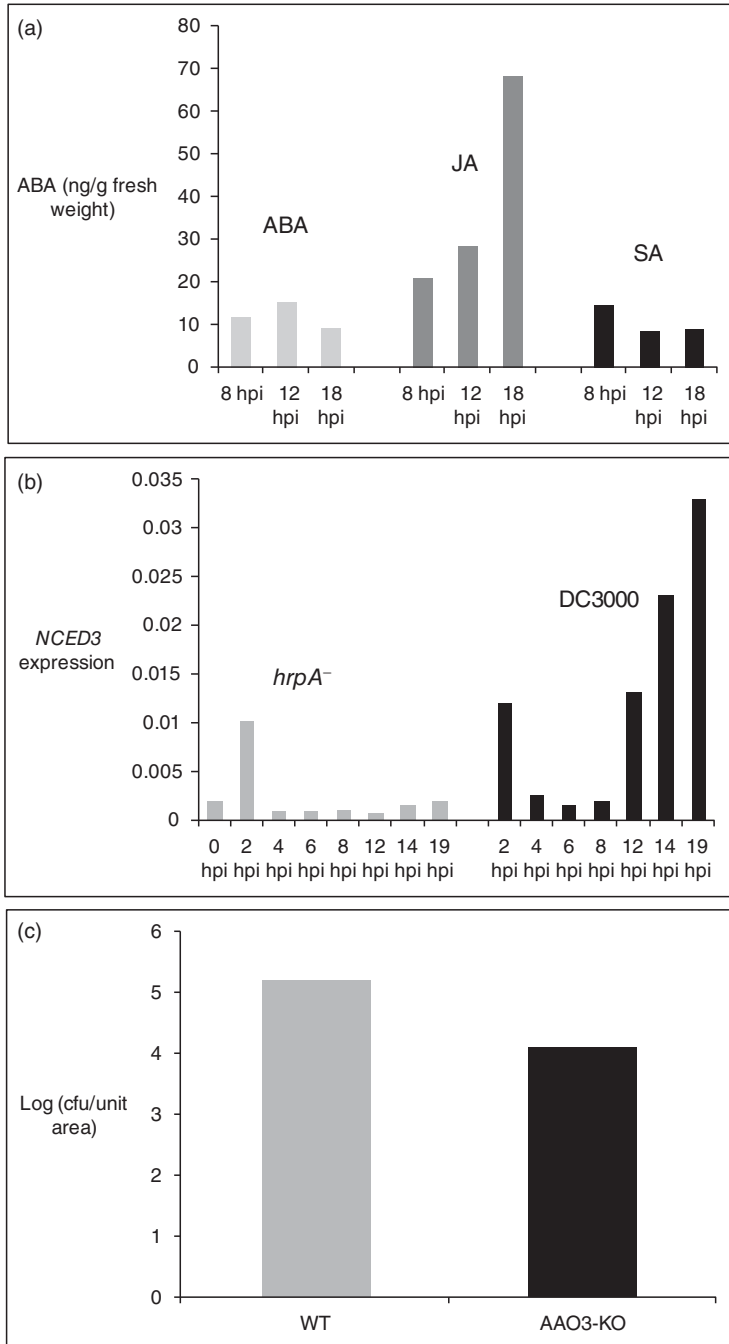


Fig. 8.3 (a) Time-course of changes in ABA levels in leaves of *Arabidopsis thaliana* (Col-5) after mock infiltration (mock) or challenge with wild type *Pseudomonas syringae* (DC3000) or a *P. syringae* mutant compromised in T3SS (*hrpA*⁻). (b) Time-course of *NCED3* expression following challenge of *A. thaliana* with *P. syringae* DC3000 or *P. syringae* DC3000*hrpA*⁻. [c] Growth of *P. syringae* DC3000 and *P. syringae* DC3000*hrpA*⁻ in leaves of wild-type *A. thaliana* (Col-0) and the ABA biosynthetic mutant of *A. thaliana*, AAO3-KO. Adapted from De Torres-Zabala *et al.* 2007. Reproduced with permission of Wiley-VCH.

virulence factors can specifically manipulate components of the ABA biosynthetic and response machinery, leading to an increase in ABA levels. The data also suggest that ABA modifies general cellular metabolic homeostasis to facilitate growth of the bacterial pathogen. Subsequent work by de Torres-Zabala *et al.* (2009) demonstrated that *Pst* effectors acted rapidly in *Arabidopsis* to activate ABA biosynthesis, the ABA then suppressing inducible innate immune responses by down-regulating SA biosynthesis and SA-mediated defences.

However, the effect of ABA is not the same in all host–pathogen interactions. For example, Fan *et al.* (2009) found that the ABA biosynthetic pathway was required for the full virulence of the obligate pathogen *Hyaloperonospora arabidopsis*, while ABA positively regulated resistance against the necrotrophic pathogen *Alternaria brassicicola*. In other work, *Arabidopsis* mutants deficient in ABA were found to be more sensitive to infection by the fungal pathogens *A. brassicicola* and *Pythium irregulare* but more resistant to *B. cinerea* (Fig. 8.4; Adie *et al.*, 2007). These results suggest that ABA is not a positive regulator of plant defence against all necrotrophic fungal pathogens. Clearly, properties of the particular host–pathogen interaction other than the pathogen’s lifestyle may be more important in determining the role of ABA in that interaction (Adie *et al.*, 2007; Fan *et al.*, 2009).

It appears clear therefore that ABA is involved in signalling in biotic stress responses. But how does ABA modulate plant defence responses? There is increasing evidence that ABA regulates defence responses through effects on callose deposition (e.g. Flors *et al.*, 2008), production of reactive oxygen species (e.g. Xing *et al.*, 2008) and regulation of defence gene expression (e.g. Adie *et al.*, 2007).

8.2.3 Auxin

Auxin plays important roles in many aspects of plant growth and development, including apical dominance, embryogenesis and cell division, expansion and differentiation (Hodson & Bryant, 2012). In order to regulate plant growth and development, auxin can induce the expression of three groups of genes: the *Aux/IAA* family, the *GH3* family, and the *SAUR* (small auxin-up RNA) family (Woodward & Bartel, 2005). In plants, most auxin occurs conjugated to amino acids, and indeed, the formation of these conjugates is an important regulatory mechanism for the activation/inactivation of indole acetic acid (IAA). Conjugation of auxin to amino acids is catalysed by IAA-amido synthetases, which are coded for by *GH3* genes (Staswick *et al.*, 2005).

8.2.3.1 Auxins and plant defence

In addition to its roles in plant growth and development, auxin is known to be involved in plant responses to biotic stress. Applied exogenously to plants, auxin has been reported to promote disease caused by various bacteria, including *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* (Yamada, 1993) and *Pst* DC3000 (Navarro *et al.*, 2006; Chen *et al.*, 2007), suggesting that auxin reduces defence responses. Interestingly, if auxin responses are blocked, disease resistance can be increased (Wang *et al.*, 2007a,b). But what happens to auxin levels and auxin signalling in plants responding to pathogen infection? Auxin levels were found to increase in *Arabidopsis* infected with *Pst* DC3000, a hemibiotrophic pathogen (O’Donnell *et al.*, 2003), while the bacterial T3E *avrRpt2* was found to modulate host auxin physiology, thereby promoting virulence of *Pst* DC3000 and disease development in *Arabidopsis* (Chen *et al.*, 2007). In the latter work, the authors used transgenic *Arabidopsis* constitutively

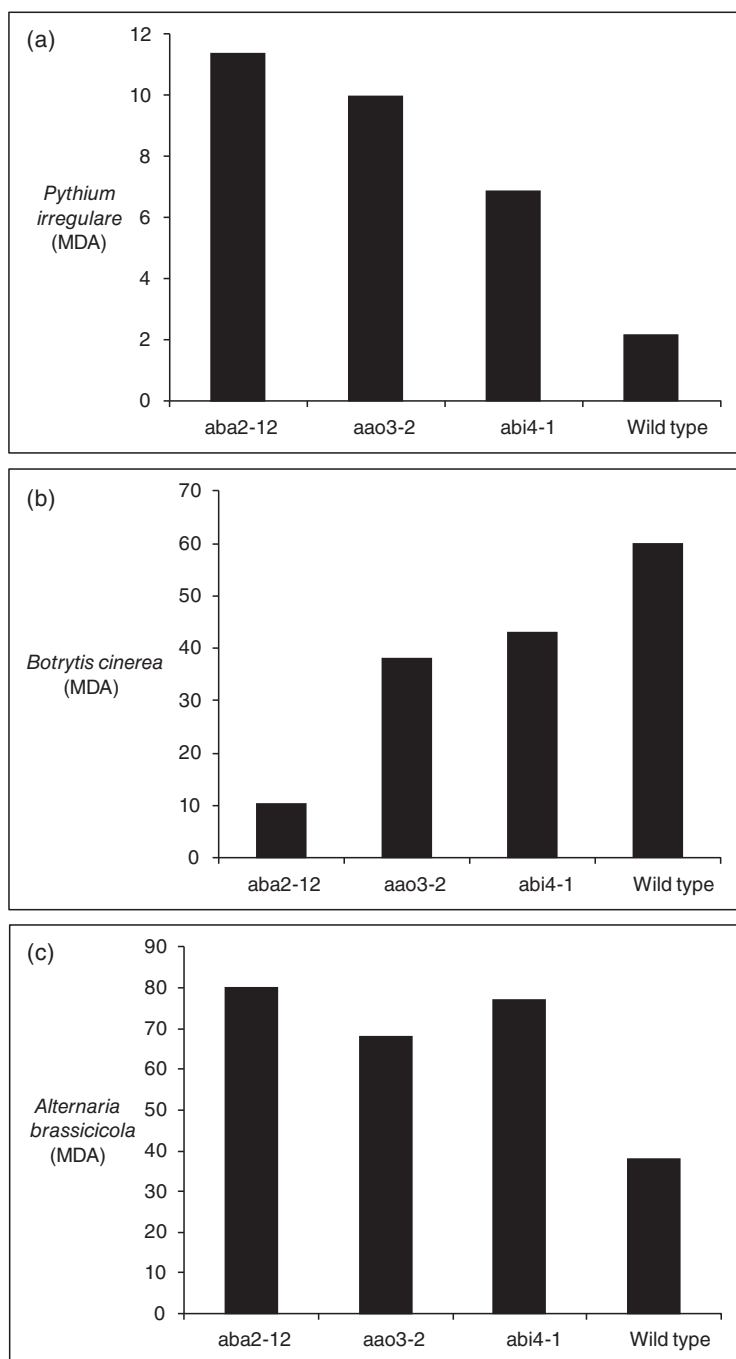


Fig. 8.4 Susceptibility of *Arabidopsis thaliana* ABA-related mutants (biosynthesis: *aba2-12* and *aao3-2*; insensitive: *abi4-1*) to infection by *Pythium irregulare* (a), *Botrytis cinerea* (b) and *Alternaria brassicicola* (c) compared to wild-type plants. MDA = mean disease area (mm²) per infected leaf, 24 hours after infection. Adapted from Adie *et al.* 2007. Reproduced with permission of American Society of Plant Biologists.

expressing AvrRpt2. The seedlings of these plants exhibited phenotypes resembling mutants with altered auxin physiology, including longer primary roots, increased number of lateral roots and increased sensitivity to exogenous auxin (Chen *et al.*, 2007). The seedlings also had increased levels of free IAA, and these were increased further during infection with *Pst* DC3000. *Pst* DC3000 infection also induced expression of genes involved in auxin biosynthesis but repressed genes belonging to the *Aux/IAA* family and auxin transporters. Chen *et al.* (2007) suggested that AvrRpt2 might be among the virulence factors used by *P. syringae* that modulate host auxin physiology in order to promote disease. In a similar vein, later work by Kidd *et al.* (2011) found that in *Arabidopsis* infected with the root-infecting hemibiotrophic fungus *Fusarium oxysporum*, genes involved in auxin biosynthesis were up-regulated in both leaves and roots, while *Arabidopsis* mutants disrupted in auxin signalling were more resistant to infection. More recent work by Evangelisti *et al.* (2013) examined the function of an effector from the oomycete pathogen *Phytophthora parasitica*. This effector, penetration-specific inhibitor 1 (PSE1), enhanced susceptibility of *Arabidopsis* to this pathogen, at least in part, by disrupting auxin accumulation in plant roots. In fact, *P. parasitica* was found to modulate auxin physiology during the first hours of infection, at the infection site.

Exactly how auxin promotes susceptibility in plants is not known, although it has been suggested that auxin suppresses SA-mediated defences (Robert-Seilanianantz *et al.*, 2011). This was tested by Mutka *et al.* (2013), who used transgenic *Arabidopsis* with enhanced auxin levels. They found that although such plants were indeed more susceptible to *Pst* DC3000, there was no impact on the ability of the plants to mount defence responses, and neither were SA accumulation and SA-responsive gene expression altered. The mechanism by which the enhanced auxin levels in these plants promoted susceptibility to *Pst* DC3000 remains to be discovered.

If auxin promotes susceptibility to pathogen infection as suggested by the work outlined previously, then perhaps disrupting auxin signalling might enhance resistance. This was examined by Navarro *et al.* (2006), who demonstrated that down-regulation of auxin receptor genes increased resistance to *Pst* DC3000 in *Arabidopsis*. It appears that many biotrophic and hemibiotrophic pathogens have evolved mechanisms to disrupt auxin homeostasis in the host. In response, the host plant could employ hormone crosstalk as a means of counteracting such pathogen-triggered disruption of auxin signalling. In fact, inhibition of auxin signalling was shown to be part of the SA-mediated host defence against biotrophic pathogens (e.g. Navarro *et al.*, 2006; Wang *et al.*, 2007a, b).

But what about necrotrophic pathogens? Work by Llorente *et al.* (2008) found that repression of auxin signalling in *Arabidopsis* compromised resistance to the necrotrophic fungal pathogens *Plectosphaerella cucumerina* and *B. cinerea* (Fig. 8.5). Interestingly, in contrast to the antagonistic crosstalk between SA and auxin during resistance to biotrophic pathogens, it was suggested that JA and auxin might interact positively in defence against necrotrophs (Kazan & Manners, 2009). This idea was supported by the work of Qi *et al.* (2012), who found that infection of *Arabidopsis* by the necrotrophic fungal pathogen *Alternaria brassicicola* up-regulated auxin biosynthesis and down-regulated auxin transport capacity, effects of which were partially dependent on JA signalling. Together, these effects led to an enhanced auxin response in the host.

In conclusion, the work described previously suggests that auxin signalling is required for resistance against necrotrophs but imparts susceptibility against biotrophs. It also appears clear that some pathogens target host auxin physiology as part of their infection strategy.

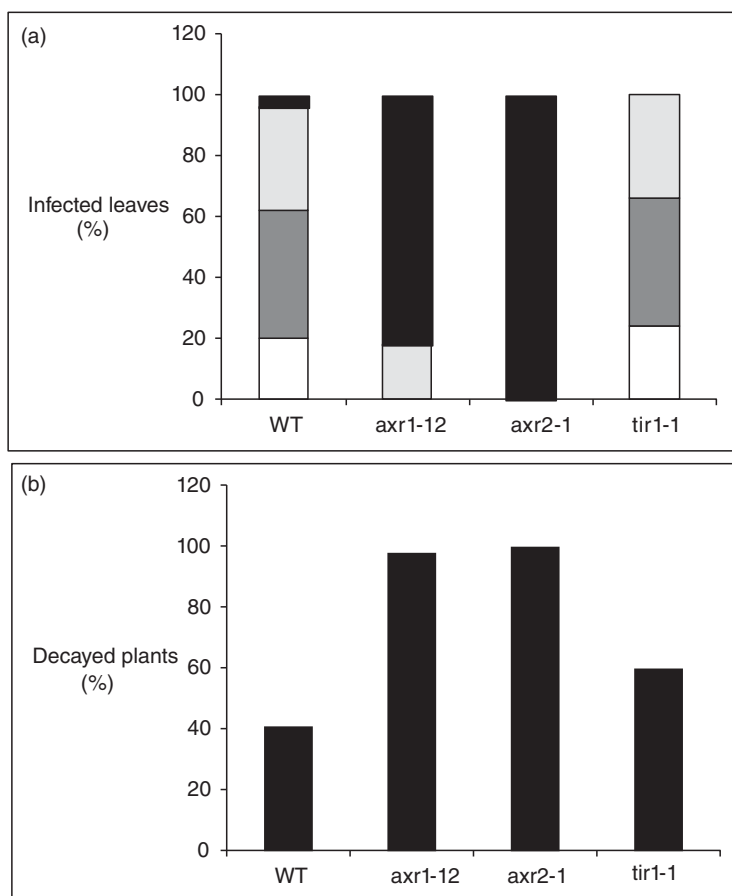


Fig. 8.5 (a) Infection of leaves of 4-week-old *Arabidopsis thaliana* plants by *Plectosphaerella cucumerina*. WT = wild type; *axr1-12*, *axr2-1*, *tir1-1* = auxin signalling mutants. Disease rating is represented as percentage of leaves showing: no symptoms (white), chlorosis (light gray), necrosis (dark gray), severe tissue maceration (black). (b) Enhanced susceptibility of auxin signalling mutants to *Botrytis cinerea*. WT = wild type; *axr1-12*, *axr2-1*, *tir1-1* = auxin signalling mutants. Percentage decay of plants was assessed 6 days after inoculation. Adapted from Llorente *et al.* 2008. Reproduced with permission of Oxford University Press.

8.2.3.2 Auxins and clubroot

Auxins are not involved only in modulation of resistance/susceptibility in plant–pathogen interactions. Changes in auxins have long been studied in relation to clubroot disease of brassicas. Clubroot disease is caused by the obligate biotrophic pathogen *Plasmodiophora brassicae*. It enters the root cortex, causing the root to swell, thereby inducing the formation of galls (or clubs). These galls represent strong metabolic sinks, and as the galls grow, they alter host metabolism, including changes in plant hormones. At the beginning of gall growth, cell division is the dominant process, whereas cell enlargement dominates the later stages of gall growth (Kobelt *et al.*, 2000). In more recent work, Malinowski *et al.* (2012) demonstrated that the main factor influencing gall size is proliferation of the vascular cambium, although there is also proliferation of the phloem parenchyma. The picture emerging from this work is that gall

formation is a consequence of pathogen-driven re-programming of existing host meristematic activity. Interestingly, the authors found that although gall formation influences the number of spores produced by the pathogen, it is not required for completion of the pathogen life cycle (Malinowski *et al.*, 2012).

The cell division and cell enlargement stages of gall growth correspond with temporal changes in plant hormones, that is with cytokinin production by *P. brassicae* plasmodia during early stages of infection (Dekhuijzen, 1981; see Section 8.2.4) and elevated auxin levels during the later stages of infection, when plasmodia are forming sporangia and resting spores (Grsic-Rausch *et al.*, 2000). Devos *et al.* (2006) found that the first sign of an auxin response occurred 5 days after inoculation in *Arabidopsis* and increased during subsequent gall development. Increases in free and conjugated IAA levels were also reported in roots of both *Arabidopsis* and *Brassica rapa* infected with *P. brassicae* (Ludwig-Müller *et al.*, 1996, 1999; Grsic-Rausch *et al.*, 2000; Devos *et al.*, 2005). IAA accumulation in galls was found to correlate with development of pathogen plasmodia in the gall (Devos *et al.*, 2006; Ludwig-Müller *et al.*, 2009), suggesting that plasmodia were accumulating IAA in a sink-dependent manner. Increased levels of IAA conjugates were also detected in *Arabidopsis* and *B. rapa* infected with the clubroot pathogen (Devos *et al.*, 2005; Ludwig-Müller *et al.*, 2009). Such an accumulation of IAA conjugates might represent an attempt to detoxify excess free IAA but might also suggest a role for IAA conjugates in gall formation (Ludwig-Müller *et al.*, 2009).

8.2.3.3 Auxins and nematodes

Plant cells infected by sedentary endoparasitic nematodes undergo a profound transformation, accompanied by a great many changes in their physiology and metabolism (Chapter 1). For example, enhanced auxin responses have been observed at infection sites of both cyst and root-knot nematodes, and mutant plants disrupted in auxin signalling have been found to be more resistant to nematode infection (Goverse *et al.*, 2000; Karczmarek *et al.*, 2004; Grunewald *et al.*, 2008). Expression of an auxin importer was found to be increased in young nematode feeding sites, suggesting that nematodes actively enhance auxin transport in affected host cells (Mazarei *et al.*, 2003). The down-regulation of an auxin efflux transporter in the initial syncytial cell (Grunewald *et al.*, 2009) would prevent the drain of auxin and thereby assist auxin import into the cells. Interestingly, Lee *et al.* (2011) found that an effector protein from the beet cyst nematode *Heterodera schachtii* modulated the activity of an auxin influx transporter in *Arabidopsis* during formation of the feeding site. These data suggest that auxin is an integral part of syncytia formation and that cyst nematodes appear to use and manipulate auxin transport and sensing mechanisms in the host during infection.

8.2.4 Brassinosteroids

BRs are structurally related to animal steroid hormones and are involved in regulating various growth and developmental responses in plants, including seed germination, cell division, flowering, reproductive development and senescence (Bajguz, 2007). BRs are also involved in abiotic stress responses in plants, and there is increasing evidence for a role for BRs in plant defence responses. They are known to enhance resistance to a range of pathogens, apparently independently of SA-mediated defence signalling (Bari & Jones, 2009). *Arabidopsis* mutants compromised in BR signalling (*bak1* mutants) showed enhanced susceptibility to necrotrophic pathogens such as *A. brassicicola* and *B. cinerea*, while resistance to the biotrophic pathogen *Hyaloperonospora parasitica* was enhanced compared to wild-type plants (Kemmerling *et al.*,

2007). More recent work has suggested that BR signalling plays an important role in modulating plant immunity during plant growth (Albrecht *et al.*, 2012; Belkhadir *et al.*, 2012). It has been proposed that biotrophic pathogens have evolved virulence mechanisms to detect or create physiological states in which BR concentrations are optimal for pathogen success, based perhaps on modifying BR biosynthesis or signalling (Belkhadir *et al.*, 2012).

8.2.5 Cytokinins

Cytokinins are involved in various plant processes ranging from vascular differentiation and chloroplast biogenesis, through to leaf senescence and stress tolerance (Muller & Sheen, 2007). They have long been studied in relation to plant disease, for example in green island formation on leaves infected with biotrophic fungi, as well as in the development of ‘clubs’ on roots of brassicas infected with *P. brassicae*.

8.2.5.1 Cytokinins and green islands

Greens island is the term used to describe rings or spots of living green tissue (usually leaf tissue) centred on a site of pathogen infection and which is usually surrounded by yellow, senescing tissue (Walters *et al.*, 2008; see also Box 8.1). They are usually associated with infection of host tissues by obligately biotrophic fungal pathogens but may also be associated with biotrophic stages of host colonisation by hemibiotrophic fungal pathogens. Whether green islands form as a result of re-greening or chlorophyll retention is still debated, but whatever the mechanism, it is clear that green island tissues are metabolically active. Although rates of net photosynthesis are reduced in leaves infected with biotrophic fungal pathogens (Chapter 3), green island tissues on such leaves are still photosynthetically active, unlike the surrounding senescing tissue. Infection sites of biotrophic fungi are also known to accumulate nutrients, as a result of increased nutrient transport towards infection sites and/or reduced transport away from these regions. Because cytokinins are known to create sink effects in plant tissue and are associated with delaying leaf senescence, they were implicated in the formation of green islands and the mobilisation of nutrients towards infection sites (Walters & McRoberts, 2006; Walters *et al.*, 2008). But is there any evidence of cytokinin involvement in these processes in interactions between plants and biotrophic/hemibiotrophic fungal pathogens? Because of the difficulty in extracting and quantifying cytokinins, early work examined ‘cytokinin-like activity’. Thus, Bushnell and Allen (1962) demonstrated that extracts of spores of the powdery mildew fungus *Blumeria graminis* were able to induce green island formation and starch accumulation on detached barley leaves, while increased cytokinin-like activity was detected in bean leaves infected with rust fungi (Kiraly *et al.*, 1966, 1967). Subsequent workers were able to quantify cytokinins, and for example, increased concentrations of the cytokinins zeatin riboside (ZR) and dihydrozeatin riboside (DHZR) were detected in leek leaves infected with rust (Roberts, 1987). Increased cytokinin levels were also found in green islands on leaves of a range of plants infected with fungal pathogens (Lopez-Carbonell *et al.*, 1998). Cytokinins were also detected in mycelial extracts and culture filtrates of the hemibiotrophic fungus *Pyrenopeziza brassicae*, as well as in mycelium of the biotrophic fungus *Cladosporium fulvum*, and in spore washings of the biotroph *B. graminis* and the hemibiotrophs *P. brassicae* and *Venturia inaequalis* (Murphy *et al.*, 1997). Interestingly, the necrotrophic fungi *B. cinerea* and *Penicillium expansum* did not produce cytokinins. These findings led Murphy *et al.* (1997) to propose a link between obligate parasitism and cytokinin production, with biotrophic and hemibiotrophic fungi secreting

cytokinins into host tissue. Subsequent work found that biotrophic and hemibiotrophic fungi, but not necrotrophs, were also able to produce enzymes capable of liberating cytokinins from cytokinin conjugates (glucosides) (Cooper & Ashby, 1998). It was suggested that cleavage of cytokinin conjugates might be used by the fungus to release stored fungal cytokinins or might be secreted at the site of infection, causing the plant to release active cytokinin from the conjugate. This would ensure accumulation of enough cytokinin at sites of infection to result in the redirection of host assimilates for pathogen growth (Cooper & Ashby, 1998). Cytokinins and cytokinin-cleaving enzymes might also contribute to green island formation on leaves infected with biotrophic and hemibiotrophic fungi (Ashby, 2000).

8.2.5.2 Cytokinins and pathogen-induced gall formation

During clubroot development in brassicas infected with *P. brassicae*, increased concentrations of free and bound cytokinins were detected (Dekhuijzen & Overeem, 1971; Dekhuijzen, 1981). Later work showed that the amount of active cytokinins such as ZR was always greater in infected *B. rapa* compared to uninfected plants, while isopentenyladenine increased in galls at 21 days after inoculation (Devos *et al.*, 2005). The situation was different in *Arabidopsis*, however, where only the isopentenyl-type cytokinins isopentenyladenine and isopentenyladenosine accumulated during infection (Devos *et al.*, 2006). Interestingly, earlier work had shown that plasmodia of *P. brassicae* were capable of making cytokinins, suggesting that at least part of the increased cytokinin content during clubroot infection might result from pathogen synthesis (Müller & Hilgenberg, 1986). Subsequent work on *Arabidopsis* found that two cytokinin oxidases/dehydrogenases were down-regulated during clubroot infection (Siemens *et al.*, 2006). Such a reduction in the capacity for cytokinin breakdown could contribute to cytokinin accumulation, especially at sites where *P. brassicae* produces cytokinins. Plants overexpressing genes responsible for cytokinin breakdown were found to be highly resistant to *P. brassicae*, highlighting the importance of cytokinins for pathogenicity in the clubroot pathogen (Siemens *et al.*, 2006).

Ustilago maydis causes common smut of maize, a disease characterised by the production of tumours in susceptible embryonic or actively growing above-ground tissues. Early work suggested that cytokinins and auxins were produced by *U. maydis* and played a role in the development of smut tumours in maize (Moulton, 1942; Mills & Van Staden, 1978). Certainly, a role for hormones in this disease appears plausible, as cytokinins and other hormones (e.g. auxin, ABA) are known to regulate source–sink relations. Indeed, IAA was reported to be made by *U. maydis* and to be released during host tumour formation, but although pathogen-produced IAA was thought to contribute to overall IAA accumulation in infected tissue, it was not thought to be responsible for triggering host tumour formation (Reineke *et al.*, 2008). More recently, Bruce *et al.* (2011) were able to identify and characterise hormones in the *U. maydis* – maize interaction and found an accumulation of both cytokinins and ABA in infected tissue. They also found that the pathogen produced both of these hormones at significant levels, making it likely that *U. maydis* was the source of these hormones in infected tissue.

8.2.5.3 Cytokinins and plant defence

Transgenic plants exhibiting elevated cytokinin levels have been found to express enhanced resistance to pathogens. For example, *Arabidopsis* plants with elevated levels of cytokinins were resistant to *Pst* DC3000 (Choi *et al.*, 2010), while tobacco plants accumulating

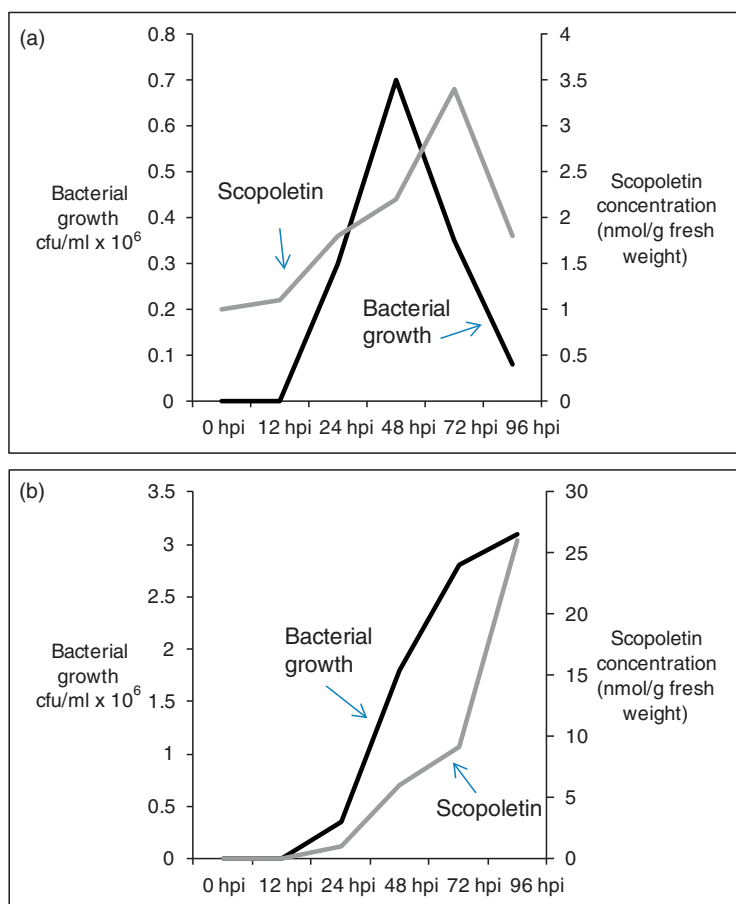


Fig. 8.6 Cytokinin-induced phytoalexin production causes pathogen resistance. (a) Time-course of scopoletin production in relation to growth of the bacterium *Pseudomonas syringae* pv. *tabaci* in tobacco leaves pre-treated with cytokinins. (b) Time-course of scopoletin production in relation to growth of the bacterium *Pseudomonas syringae* pv. *tabaci* in tobacco leaves not treated with cytokinins. Note the different scale for scopoletin levels in (a) and (b). Adapted from Großkinsky *et al.* Reproduced with permission of American Society of Plant Biologists.

cytokinins exhibited enhanced resistance to *P. syringae* pv. *tabaci* (Großkinsky *et al.*, 2011). In the latter work, the cytokinin-mediated resistance was strongly correlated with up-regulated synthesis of two phytoalexins, scopoletin and capsidiol (Fig. 8.6). It is noteworthy that this cytokinin-mediated resistance in tobacco is independent of SA and JA but occurs via a rapid accumulation of phytoalexins early in attempted infection, resulting in effective restriction of pathogen growth. Recent work on rice found that cytokinins accumulated after infection with the hemibiotrophic rice blast pathogen *Magnaporthe grisea*, and cytokinin signalling was activated around infection sites (Jiang *et al.*, 2013). It was also found that *M. grisea* was capable of producing cytokinins, although it appeared likely that the massive accumulation of cytokinins was of host origin. Jiang *et al.* (2013) also found that cytokinins and SA acted synergistically to activate defences, leading them to suggest that during evolution of this interaction, *M. grisea* would elevate cytokinin levels as a means of attracting nutrients, while

the host plant would sense the increased cytokinin, and synergistically with SA, activate defence responses.

Box 8.1 Polyamines and plant disease

Polyamines are a group of simple aliphatic amines, the most common of which are the diamine putrescine, the triamine spermidine and the tetraamine spermine. They are essential for growth of most organisms, and among their various functions in plants is the regulation of senescence (Takahashi & Kakehi, 2010). Polyamines were shown to accumulate in leaves infected with obligately biotrophic fungal pathogens, such as rusts and powdery mildews (e.g. Greenland & Lewis, 1984; Walters *et al.*, 1985; Walters & Wylie, 1986; Foster & Walters, 1992) and were also found to accumulate in green islands on powdery-mildew-infected barley leaves (Coghlan & Walters, 1990). It had been suggested that the accumulation of polyamines in diseased leaves, and especially in association with green islands, was related to the well-known senescence retarding effects of polyamines. Indeed, in the work of Coghlan & Walters (1990), polyamine accumulation was associated with reduced activity of lipoxygenase and greatly reduced ET evolution, suggesting that membrane damage in green island tissue was minimal and senescence was delayed.

Polyamines are also known to be important in plant responses to abiotic and biotic stress (Walters, 2003a, 2003b; Alcázar *et al.*, 2010). In particular, increased polyamine biosynthesis and polyamine levels were found in plant tissues exhibiting a hypersensitive reaction (HR) to attempted infection, for example in tobacco displaying a HR to Tobacco Mosaic Virus (TMV) (e.g. Torrigiani *et al.*, 1997; Yamakawa *et al.*, 1998) and in the HR of barley to the powdery mildew fungus, *B. graminis* f.sp. *hordei* (Cowley & Walters, 2002). In the barley HR response, not only was polyamine biosynthesis increased, but also was polyamine catabolism, in particular activities of the enzymes diamine oxidase (DAO) and polyamine oxidase (PAO). The breakdown of polyamines by DAO and PAO leads to the formation of hydrogen peroxide, and it was suggested that this might be one of the signals responsible for triggering the HR (Walters, 2003b). Interestingly, in later work, Moschou *et al.* (2009) demonstrated that tobacco plants overexpressing PAO were more tolerant of infection by the biotrophic *P. syringae* pv. *tabaci* and the hemibiotrophic *P. parasitica* var. *nicotianae*. In *Arabidopsis*, overexpressing the gene responsible for biosynthesis of spermine (spermine synthase) led to accumulation of spermine and increased resistance to *Pseudomonas viridiflava*. This increased resistance was compromised if transgenic plants were treated with a PAO inhibitor, suggesting that the increased resistance was the result, at least in part, of spermine oxidation (Gonzalez *et al.*, 2011).

However, the involvement of polyamines in plant resistance to pathogens is not as straightforward as the previous section might suggest. Thus, in tomato overexpressing the gene for spermidine synthase, the resulting spermidine accumulation was associated with decreased resistance to the necrotrophic fungus *B. cinerea* (Nambeesan *et al.*, 2012). The up-regulation of spermidine biosynthesis was associated with a down-regulation of ET biosynthesis and signalling, leading the authors to suggest that the polyamine-mediated susceptibility of tomato to *B. cinerea* might have been the result of interference with the functions of ET in plant defence.

8.2.6 Gibberellins

Originally identified as a substance secreted by the fungus *Gibberella fujikuroi*, the cause of 'foolish seedling' disease of rice, gibberellins (GAs) are a group of isoprenoid hormones involved in regulating many aspects of plant growth and development, including seed germination, stem elongation and flowering. GAs promote plant growth by stimulating breakdown of negative regulators of growth known as DELLA proteins. In *Arabidopsis*, DELLA proteins were found to control plant immune responses by modulating SA- and JA-dependent defence responses (Navarro *et al.*, 2008). An *Arabidopsis* mutant deficient in four *DELLA* genes was found to be susceptible to the fungal necrotrophs *B. cinerea* and *A. brassicicola*, but more resistant to the biotrophs *Pst* DC3000 and *Hyaloperonospora arabidopsidis*. When this mutant was inoculated with *Pst* DC3000, there was more rapid and stronger induction of SA marker genes and delayed expression of a JA/ET marker gene compared to wild-type plants (Navarro *et al.*, 2008). In contrast, *DELLA* over-accumulating mutants were more resistant to *A. brassicicola* and more susceptible to *Pst* DC3000. Taken together, these results suggest that *DELLA* proteins promote resistance to necrotrophic pathogens by activating JA/ET-mediated defences but increase susceptibility to biotrophs by repressing SA-mediated defences.

Rice mutants defective in GA perception, such as the *gid1* mutant, have also been shown to express altered defence responses. The *gid1* mutant accumulates GA and is more resistant to *M. grisea* than wild-type plants (Tanaka *et al.*, 2006). In addition, altering GA levels by manipulating the activities of GA deactivating enzymes can also influence defence responses. One such enzyme is 'Elongated Uppermost Internode' (EUI) and loss of function *eui* rice mutants accumulate high levels of GA and exhibit compromised resistance, while EUI overexpressors accumulate low GA levels and show enhanced resistance to the rice pathogens *Xanthomonas oryzae* pv. *oryzae* and *M. grisea* (Fig. 8.7; Yang *et al.*, 2008). It was suggested that GAs play a negative role in basal disease resistance in rice.

8.2.7 Conclusion

We have seen that interactions between SA and JA/ET are at the core of plant immunity. However, this core can be influenced by other hormones, including ABA, auxin, cytokinins and GAs, thereby affecting the outcome of a plant–pathogen interaction. One example of this complex network of regulation in plant defence is the antagonism between auxin and cytokinin. Naseem and Dandekar (2012) examined this antagonism using the interaction between *Arabidopsis* and *Pst* DC3000 as a model. In this system, auxin inhibits SA responses and in so doing, indirectly promotes the effect of JA signalling in defence, while cytokinin reinforces SA responses. This hormonal interplay exerts a critical influence on the final outcome of a host–pathogen interaction but is, at present, little understood. Naseem and Dandekar (2012) advocate a systems biology approach to analyse the impact of hormonal interactions in plant immunity (see also Box 8.2). Given the complexity of such interactions, a systems biology approach appears sensible to help increase our understanding of how plants regulate defence.

Box 8.2 Beating the clock! The circadian clock and plant immunity

Many plant processes are regulated by circadian rhythms, including hypocotyl extension, stomatal opening and gene expression. Importantly, rhythmic activity continues even if plants are moved from daily cycles of light and dark to continuous darkness or continuous light. These are known as free running rhythms. In fact, plants contain an internal system, called the circadian clock, capable of measuring 24-hour time intervals and generating these rhythms. In plants, the system that generates circadian rhythms can be split into three parts: (i) a component comprising input pathways involved in transmitting environmental signals to the circadian clock, (ii) the circadian clock itself, and (iii) a part composed of a variety of rhythmic outputs (e.g. gene expression) that are controlled by the circadian clock (Smith *et al.*, 2010). Two core components of the clock, CIRCADIAN CLOCK ASSOCIATED1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY), are transcription factors involved in regulating clock activity (e.g. Lu *et al.*, 2009). The circadian clock is known to enhance plant fitness (e.g. Michael *et al.*, 2003; Graf *et al.*, 2010).

There have been several reports that plant defence gene expression is regulated by circadian rhythms, leading to the suggestion that plant innate immunity is controlled by the circadian clock (e.g. Weyman *et al.*, 2006; Roden & Ingle, 2009). For example, under free running conditions, *Arabidopsis* plants exhibit temporal oscillations in susceptibility to infection by *Pseudomonas syringae*, which are perturbed in plants overexpressing CCA1 (Bhardwaj *et al.*, 2011). In addition, altered expression of various clock genes, including CCA1, increases susceptibility of *Arabidopsis* to *P. syringae* and *H. arabidopsidis* (Bhardwaj *et al.*, 2011; Wang *et al.*, 2011). Later work found that disrupting the circadian clock by overexpression of CCA1 or LHY led to severe susceptibility to *P. syringae* (Zhang *et al.*, 2013). This work also demonstrated that the defence role of these two genes is partly via circadian control of stomatal aperture, although it is independent of SA-mediated defence. Interestingly, Zhang *et al.* (2013) found that infection of *Arabidopsis* with *P. syringae* shortened the circadian period, suggesting that defence activation can serve as both an input signal to regulate the clock and an output of the circadian clock.

The work of Zhang *et al.* (2013) also reveals crosstalk between the circadian clock and plant innate immunity in *Arabidopsis*. Is such crosstalk likely to be advantageous to the plant? Regulation of plant defence by the circadian clock suggests that timing of defence responses is important for plant fitness under attack. We know, however, that defence is costly to the plant, and regulation of the circadian clock by defence activation could be a means of balancing growth and development on the one hand and defence on the other hand. In case you are wondering about the relevance of the circadian clock in a chapter on hormones, several hormones interact with the circadian clock. Thus, auxin regulates clock activity as an input (Hanano *et al.*, 2006), and auxin production and signalling are affected by the clock (Rawat *et al.*, 2009). In addition, ABA, BRs, cytokinins and GAs serve as clock inputs, while ET and JA production and signalling are on the output pathways for the clock (e.g. Thain *et al.*, 2004; Hanano *et al.*, 2006; Robertson *et al.*, 2009; Goodspeed *et al.*, 2012). At present, how these hormones interact in clock-defence crosstalk is not known, but such information could prove useful in our attempts to enhance crop protection.

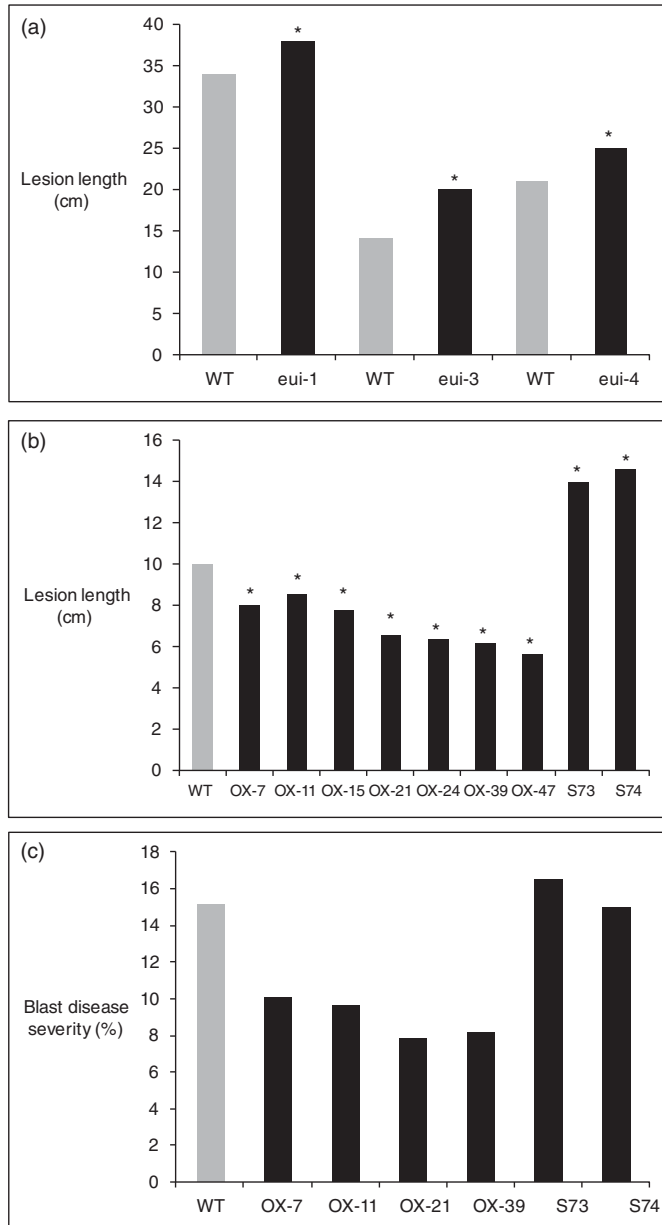


Fig. 8.7 (a) Enhanced susceptibility of *eui* gibberellin mutants to *Xanthomonas oryzae* pv. *oryzae*. WT = wild type. Three alleles of *eui* mutations were studied: *eui-1* and the wild-type ZS97, *eui-3* and the wild-type ZH11, and *eui-4* and the wild type 02428. * = significantly different from the respective WT. (b) Enhanced resistance of *Eui* overexpressors to *X. oryzae* pv. *oryzae*. OX = *Eui* overexpressors; S73 and S74 = RNAi mutants in which *Eui* expression was knocked down. * = significantly different from the WT. (c) Enhanced resistance of *Eui* overexpressors to *Magnaporthe oryzae*. OX = *Eui* overexpressors; S73 and S74 = RNAi mutants in which *Eui* expression was knocked down. Adapted from Yang *et al.* 2008. Reproduced with permission of Oxford University Press.

8.3 HORMONAL CHANGES IN PLANTS RESPONDING TO INSECT ATTACK

Much is known about JA and plant defence responses to insect attack, and although considerably less is currently known about the impact of other hormones, the situation is changing. As might be expected, hormones are not involved only in defence, and in the following section, we will examine the involvement of hormones in, for example, gall formation in insect–plant interactions.

8.3.1 Absciscic acid

ABA levels have been shown to increase in plants attacked by insects, as well as in plants treated with insect oral secretions (OSs). For example, ABA levels increased in goldenrod (*Solidago altissima*) attacked by caterpillars of tobacco budworm (*Heliothis virescens*), as well as in maize attacked by the western corn rootworm, *Diabrotica virgifera virgifera* (Erb *et al.*, 2009, 2011; Tooker & De Moraes, 2011). Root herbivory by *D. v. virgifera* resulted in water loss and increased ABA levels in roots and especially shoots (Erb *et al.*, 2009, 2011). Shoots of these plants were more resistant to herbivory by *Spodoptera littoralis* caterpillars, and the increased ABA was found to be partly responsible for the increased defences detected (Erb *et al.*, 2011). Interestingly, however, the increased ABA and the associated ABA-induced defences were not responsible for the increased resistance of the shoots. Instead, Erb *et al.* (2011) found that hydraulic changes in the leaves, induced by root herbivory, were crucial for the increased insect resistance in the shoot. The finding that herbivory-induced ABA accumulation in maize was not responsible for the increased resistance to shoot herbivory by *S. littoralis* was unexpected, especially because *Arabidopsis* mutants deficient in ABA were found to be highly susceptible to *S. littoralis* (Bodenhausen & Reymond, 2007). In fact, ABA is known to affect herbivore-induced gene expression and biosynthesis of JA in *Arabidopsis*, the induction of JA-mediated defences in maize and resistance to insect herbivory in tomato (Thaler & Bostock, 2004; Adie *et al.*, 2007; Bodenhausen & Reymond, 2007; Erb *et al.*, 2009). There is clearly crosstalk between JA and ABA, and much data suggests that regulation of ABA levels in plants responding to herbivore attack can modulate JA-mediated defences. What is not known, however, is the extent to which ABA is involved in recognition-mediated responses to herbivore attack, especially because insect damage often affects plant water relations, resulting in altered ABA levels. It is interesting to note therefore that recent research by Dinh *et al.* (2013) supports a role for ABA in plant defence against insect attack. These workers identified a novel protein, HERBIVORE ELICITOR REGULATED1 (HER1), which is part of the pathway involved in transducing signals from insect OSs. *Nicotiana attenuata* plants silenced in the expression of this protein had greatly reduced resistance against the insect herbivore *M. sexta*, together with reduced defences. These plants were also drought sensitive and displayed reduced ABA levels in leaves, suggesting that silencing the HER1 protein interfered with ABA metabolism. On the basis of these results, Dinh *et al.* (2013) suggest that HER1 suppresses ABA catabolism after herbivore attack, thereby allowing activation of the full defence profile of the plant.

8.3.2 Auxin

8.3.2.1 Auxin and defence against insect herbivores

We saw earlier in this chapter that plant resistance to pathogens can be modulated through changes in auxin sensitivity. Auxin is known to directly suppress SA-induced defences (Wang *et al.*, 2007a, b) and could potentially affect SA-mediated resistance to phloem-feeding insects such as aphids (Li *et al.*, 2006). What is not known at present, however, is whether such insects can alter auxin homeostasis or signalling as a means of suppressing host defences. There also appears to be an intimate molecular interplay between auxin and JA signalling, with data suggesting that herbivore-induced JA can affect auxin homeostasis (Pauwels *et al.*, 2009; Onkokesung *et al.*, 2010). Much remains to be discovered concerning the involvement of auxin in plant defence against insect herbivores, but evidence so far indicates that auxin modifies herbivore-relevant defence responses, and it has been suggested that plants might modulate auxin levels as a means of mediating attacker specificity (Erb *et al.*, 2012).

Auxin and JA have also been found to play a role in mediating the outcome of resource conflicts facing plants responding to insect attack. When leaves of *N. attenuata* were attacked by caterpillars of *M. sexta*, sugar and starch concentrations were decreased in roots, and regrowth from the rootstock, as well as flower production, was reduced (Machado *et al.*, 2013). The major regulators of this root-mediated resource-based trade-off were found to be jasmonates, as lower jasmonate levels were associated with decreased defence, increased levels of carbohydrates and improved regrowth from the rootstock. Auxin was found to accumulate rapidly in leaves and roots of attacked plants. Moreover, inhibiting auxin transport ameliorated the herbivore-induced reduction in regrowth. These and other data suggest that auxin homeostasis is important in determining plant tolerance against insect herbivory. It appears therefore that both JA and auxin regulate trade-offs between induced defences and tolerance in *N. attenuata* (Machado *et al.*, 2013).

8.3.2.2 Auxin and gall formation

Some insects appear to have evolved the ability to manipulate plant hormones for their own benefit, and some are even thought to synthesise plant hormones (Hori, 1992; Mapes & Davies, 2001a, 2001b; Tooker & De Moraes, 2005; Giron *et al.*, 2007). Insects possessing these abilities include gall-inducing species, which coerce the plant into producing galls that provide the insects with food and shelter. Evidence suggests that this coercion (or hijacking of the plant's physiology) involves plant hormones, including the auxin IAA. Indeed, IAA was detected in some gall-inducing insect larvae, as well as in their salivary secretions, leading to the suggestion that these insects can synthesise IAA (Hori, 1992; Mapes & Davies, 2001a). However, as Tooker and De Moraes (2011) point out, IAA found in insects and their salivary secretions might have been accumulated during feeding.

The involvement of IAA in gall formation was examined in detail by Tooker and De Moraes (2011). They used caterpillars of *Gnorimoschema gallaesolidaginis* (*G.s.*), which induces spindle-shaped galls on stems of *Solidago altissima*. This species is unusual in that, unlike other caterpillar species, it does not alter levels of JA or volatiles in its host, nor does

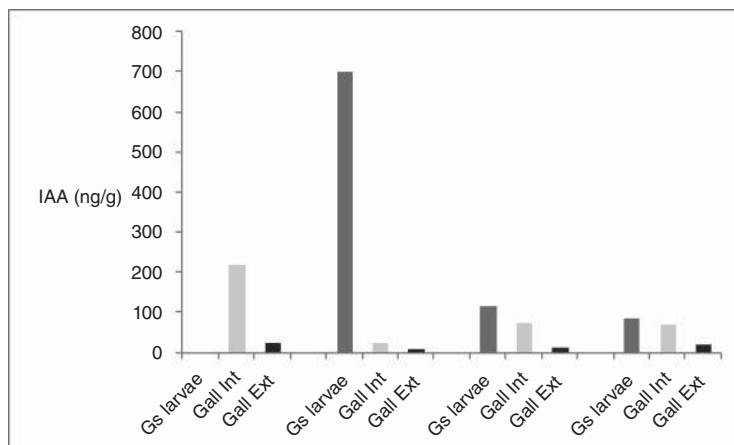


Fig. 8.8 IAA levels in interior and exterior tissue from galls induced on *Solidago altissima* by larvae of *Gnorimoschema gallaesolidaginis*. Galls were classified into four different diameter sizes: class A = 2.75–6.69 mm; class B = 6.69–10.63 mm; class C = 10.63–14.57 mm; class D = 14.57–18.50 mm. Caterpillar larvae were recovered from galls in classes B, C and D, but not from class A. Adapted from Tooker & De Moraes 2011. Reproduced with permission of Springer Science + Business Media.

it alter levels of SA. *G.s* caterpillars were found to alter IAA content of galls relative to ungalled stems, and the larvae, especially young larvae, contained high concentrations of IAA (Fig. 8.8). Interestingly, however, the generalist caterpillar *Heliothis virescens* caused no change in IAA content of plant tissue, nor did it accumulate significant concentrations of IAA, suggesting that the specialist *G.s* exerts a greater influence over its host's physiology than generalist insect species. Tooker and De Moraes (2011) suggest that because of the high concentrations of IAA in *G. s* caterpillars, they might be the source of the IAA in galls, as previously suggested by Mapes and Davies (2001a). ABA can increase after insect herbivory and can act independently of JA to induce plant defences (e.g. Bostock, 1999; Erb *et al.*, 2009). Tooker and De Moraes (2011) found that *G. s* avoided triggering increases in ABA levels in its host and might even have suppressed ABA levels. They suggest that perhaps the gall insects initially evolved an ability to manipulate IAA levels as a means of countering plant defences and that the IAA-induced gall formation that resulted was simply a secondary benefit.

8.3.3 Cytokinins

8.3.3.1 Cytokinins and defence against insect herbivory

A role for cytokinins in plant–insect interactions is suggested by the fact that when *N. attenuata* is treated with insect-derived elicitors, expression of cytokinin-related genes is greatly affected (Hui *et al.*, 2003; Gilardoni *et al.*, 2010). Indeed, tobacco plants overexpressing the cytokinin biosynthetic enzyme isopentenyltransferase exhibit enhanced resistance to the insect herbivore *M. sexta* (Smigocki *et al.*, 1993). Cytokinins also appear to activate JA biosynthesis. Thus, plants with elevated cytokinin levels exhibit increased rates of JA production after wounding, while cytokinin treatment leads to increased wound-induced JA levels and expression of genes involved in JA formation (Sano *et al.*, 1996; Dervinis *et al.*, 2010). In view of the fact that cytokinins can modulate defences induced by insect herbivores, and that cytokinin

levels appear to be dependent on ontogeny, it has been suggested that the intensity of defence responses in a particular tissue might depend on the cytokinin status of the tissue (Ballare, 2011; Erb *et al.*, 2012).

8.3.3.2 Cytokinins and interactions with leaf-mining and gall forming insects

Gall-inducing insects and leaf-mining insects have an intimate relationship with the host plant, often involving active manipulation of the host's physiology and morphology. Among the changes occurring in host tissues are the formation of 'green-islands' and the manipulation of source–sink relationships, resulting in movement of nutrients towards insect feeding sites. As with green-island formation on leaves infected with biotrophic fungal pathogens (Section 8.2.5.1), cytokinins have long been implicated in the formation of green-islands associated with the activity of leaf-mining lepidopterans and in green-islands surrounding the galls of various insects (Engelbrecht *et al.*, 1969; Engelbrecht, 1971; Elzen, 1983; Mapes & Davies, 2001a). In apple infested with the leaf miner *Phyllonorycter blancardella*, increased levels of the cytokinins zeatin, isopentenyladenine and isopentenyl adenosine were detected in green-islands compared to other parts of the leaf or non-infested leaves (Fig. 8.9; Giron *et al.*, 2007). This accumulation of cytokinins would explain both the formation of green-islands and the accumulation of nutrients in mined tissues, the latter occurring in a pattern that matched the energy requirements of the developing insect larvae (Giron *et al.*, 2007). Cytokinins have been found in a wide range of galling insects (Elzen, 1983), as well as in the leaf miner *P. blancardella*, leading to the suggestion that the accumulated cytokinins found in apple leaves infested with *P. blancardella* could have originated from the insect itself (Giron *et al.*, 2013). Interestingly, it was discovered that *Wolbachia* bacteria are involved in formation of green-islands induced on apple leaves by *P. blancardella* (Kaiser *et al.*, 2010). Since bacteria such as these can produce cytokinins, it is possible that the cytokinins in the insect might have been produced by its endosymbiont bacteria (Giron *et al.*, 2013). Indeed, subsequent work by Body *et al.* (2013) found that not only was the insect, via its endosymbiont, modifying the cytokinin profiles of the host, but also it was doing so in both senescing (photosynthetically inactive) and normal (photosynthetically active) leaf tissues (Fig. 8.10). Moreover, only insect larvae harbouring bacterial endosymbionts contained significant amounts of cytokinins, and these were most likely not derived from the host plant. It is worth mentioning in this case that endosymbiotic *Wolbachia* are also associated with the western corn rootworm *D. v. virgifera* and have been shown to play an important role in down-regulating the insect-induced plant defences (Barr *et al.*, 2010). Whether this involves cytokinins originating from the endosymbiont is not known.

8.4 HORMONAL CHANGES IN PLANTS INFECTED WITH PARASITIC PLANTS

8.4.1 Absciscic acid

Parasitic plants are known to accumulate large quantities of ABA. For example, ABA levels in *Striga* growing on maize were an order of magnitude greater than levels in the host, which were increased by some 60% after infection by the parasite (Fig. 8.11; Taylor *et al.*, 1996). It was suggested that water deficit around the parasite haustorium might have stimulated

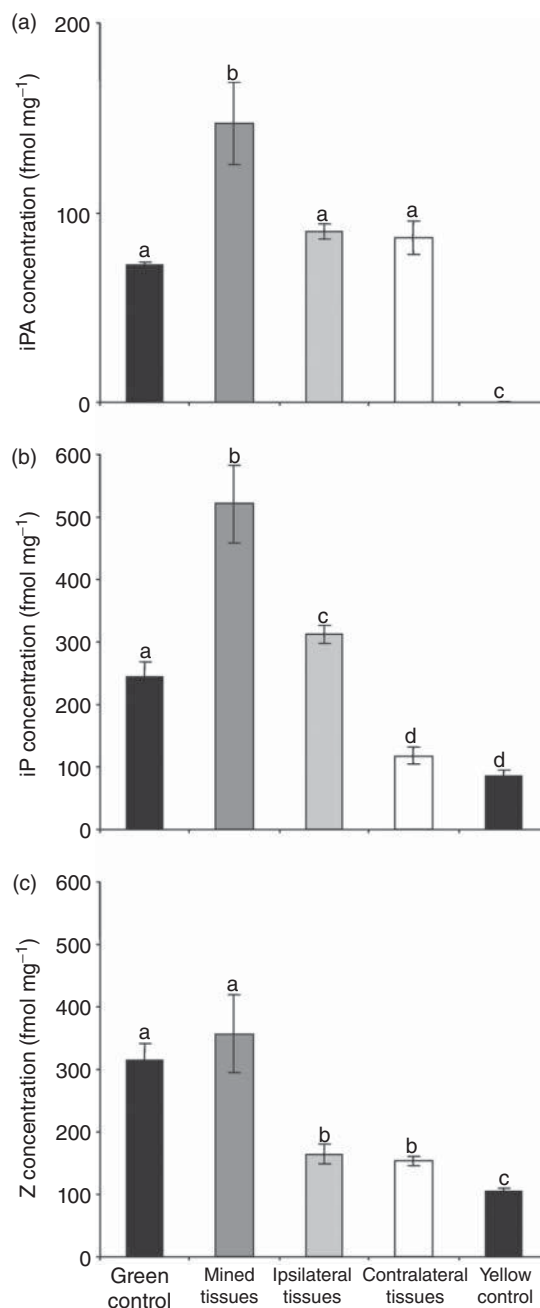


Fig. 8.9 Spatial changes in the levels of cytokinins of senescing apple leaves. (a) Isopentenyladenosine, (b) isopentenyladenine and (c) zeatin concentrations in mined, ipsilateral and contralateral plant tissues are expressed as means $\pm$ s.e. Non-infected green or yellow apple leaves were used as a control. Homologous groups are indicated by identical characters (Kruskal–Wallis test: iP, $p < 0.01$; iPA, $p < 0.05$; Z, $p < 0.01$). Ipsilateral tissues = leaf tissues situated on the same side of the main vein as the mine; contralateral tissues = leaf tissues on the opposite side of the main vein as the mine. Giron 2007. Reproduced with permission of Royal Society Publishing.

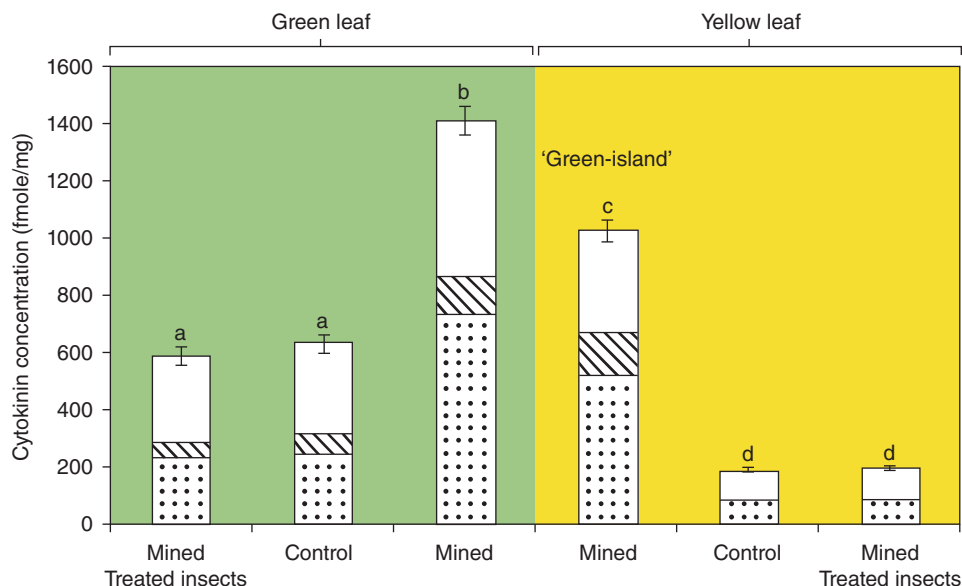


Fig. 8.10 Effects of leaf-mining by on levels of cytokinins in apple tree leaves. Non-mined green or yellow apple tree leaves were used as a control. Statistical differences between means are shown by different letters. Isopentenyladenine = dotted patterns, isopentenyl adenosine = striped patterns, zeatin = white. Treated insects = larvae treated with antibiotics to remove endosymbiotic bacteria. Body *et al.* 2013. Reproduced with permission of Springer Science + Business Media.

ABA formation in maize roots. ABA concentrations were also found to be increased in xylem sap and leaves of sorghum infected with *Striga*, leading to the suggestion that the elevated ABA might be involved in regulating stomatal conductance (Frost *et al.*, 1997; Drennan & El Hiweris, 1979). In the root hemiparasite *Rhinanthus minor*, not attached to a host, ABA concentrations in roots and shoots were 15 times greater and in xylem sap 10 times greater than in its barley host (Jiang *et al.*, 2004). As with the *Striga*/maize interaction (Taylor *et al.*, 1996), ABA concentrations in leaves of *R. minor* attached to its host were an order of magnitude greater than concentrations found in host leaves. However, ABA concentrations in the parasitized host were not significantly different from unparasitised plants (Jiang *et al.*, 2004). The reason for the extremely high ABA concentration in attached *R. minor* is not known. As Jiang *et al.* (2004) point out, it appears unlikely that it is related to stomatal function, as stomata of the parasite remain continuously open, even at night.

8.4.2 Auxin

It is generally accepted that localisation and orientation of vascular tissues are determined by IAA (Sachs, 1981; Reinhardt *et al.*, 2003; Scarpella *et al.*, 2006). In the early stages of infection by a parasitic plant, there is fusion of the vascular systems of host and parasite, and the connection and symplastic continuity between the two partners have been described in detail (e.g. Dörr & Kollman, 1995; Dörr, 1996). It appears reasonable to assume therefore that IAA might be involved in infection of the host by a parasitic plant. Indeed, in the facultative hemiparasite *Triphysaria versicolor*, auxin concentration was found to be critical for parasite attachment to

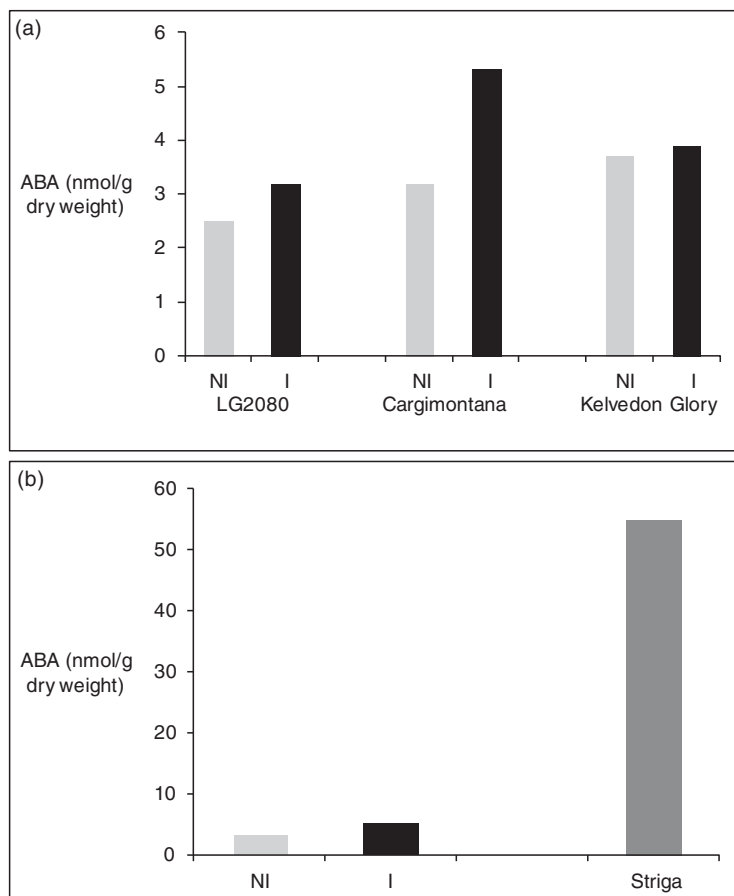


Fig. 8.11 (a) ABA concentration in leaf tissue of the youngest fully expanded leaf of three varieties of maize (LG2080, Cargimontana, Kelvedon Glory), 45 days after planting. NI = not infected; I = infected with *Striga hermonthica*. (b) ABA concentration in shoot tissue of *S. hermonthica* (Striga) compared to leaf tissue of the maize cultivar Cargimontana. NI = not infected; I = infected with *S. hermonthica*. Taylor *et al.* 1996. Reproduced with permission of Oxford University Press.

the host (Tomilov *et al.*, 2004, 2005). In this work, haustorium formation by the parasite was associated with IAA accumulation, and haustorium formation could be reversibly inhibited using auxin transport inhibitors. In the interaction between *Orobanchae aegyptiaca* and its host, *Arabidopsis thaliana*, disrupting auxin transport prevented host infection by the parasite (Bar-Nun *et al.*, 2008). In this case, it appeared that auxin flow from the host to the parasite was essential in establishing the linkage between the vascular systems of host and parasite.

8.4.3 Cytokinins

Changes in cytokinin levels have been detected in interactions between parasitic plants and their hosts. For example, attachment of *Striga hermonthica* to sorghum reduced cytokinin levels in host xylem sap by 91–97% and in leaves by 70–99% (Drennan & El Hiweris, 1979).

In the interaction between *R. minor* and its barley host, infection reduced synthesis of the cytokinin zeatin in barley roots by 57% and decreased flow of zeatin the host xylem (by 56%) and decreased zeatin metabolism in barley leaves (Jiang *et al.*, 2005). In contrast, flow of zeatin in host phloem was increased threefold. After attachment to the host, zeatin content in roots of the parasite increased, as did flows of the cytokinin in the xylem and phloem (by 20-, 12-, and 29-fold, respectively). Since net synthesis of zeatin in roots of the parasite was reduced by some 35%, a large proportion of the xylem flow of zeatin (70%) came from the host. Cytokinins can act as antagonists of ABA on stomatal behaviour (e.g. Blackman & Davies, 1984; Mansfield & McAinsh, 1995). Stomata of *R. minor* remain open despite accumulating very large concentrations of ABA (Jiang *et al.*, 2004), and it has been suggested that the high amounts of cytokinins arriving in the apoplast of *R. minor* leaves via the xylem might antagonise ABA action, thereby keeping stomata open (Jiang *et al.*, 2005).

8.4.4 Gibberellins

Information on gibberellins in interactions between parasitic plants and their hosts is scant. Nevertheless, it is known that clover broomrape (*Orobanche minor*) can synthesize some minor gibberellins, but is likely to obtain most of the gibberellins it requires from its host (Suzuki *et al.*, 1994). In contrast, the parasitic plant *Aeginetia indica* is capable of producing most of its gibberellins and is therefore likely to be independent of its *Miscanthus sinensis* host in terms of a gibberellin requirement (Suwa *et al.*, 1995).

8.4.5 Salicylic acid and jasmonic acid

In a study of signalling in the interaction between tomato and the parasitic plant *Cuscuta pentagona*, attachment of the parasite to a 10-day-old host elicited few changes, whereas a second attachment 10 days later led to a 60-fold increase in JA and a 30-fold increase in SA, accompanied by a hypersensitive-like response (Runyon *et al.*, 2010). Interestingly, the parasite grew larger on hosts deficient in SA or insensitive to JA, suggesting that both hormones mediate defences against this parasite in tomato. Since levels of JA peaked 12 hours before SA (Fig. 8.12), it appeared likely that tomato defences were coordinated by sequential induction of JA and SA. The second attachment by the parasite was also accompanied by increased ABA levels (Fig. 8.12), possibly reflecting water stress induced by parasite removal of water from the host. ABA content of the parasite was considerably greater than that of the host, and although its function in the parasite is not known, it has been suggested that perhaps the ABA might increase flow of water and nutrients to the parasite (De Bock & Fer, 1992; Taylor *et al.*, 1996) or it might function in defence modulation (Adie *et al.*, 2007; Bodenhausen & Reymond, 2007).

8.5 CONCLUSIONS

As we have seen in this chapter, hormones can change in attacked plants for various reasons. Hormone changes might reflect host responses to water stress induced as a result of attack, or they might reflect changes in host growth and morphology, such as gall formation. However, hormonal changes might also signify an intricate interplay of host defence and parasite attack. We know that, depending on the nature of the attacker, defences might be mediated by SA or JA/ET and are likely to be influenced by changes in other hormones, such as ABA, cytokinins

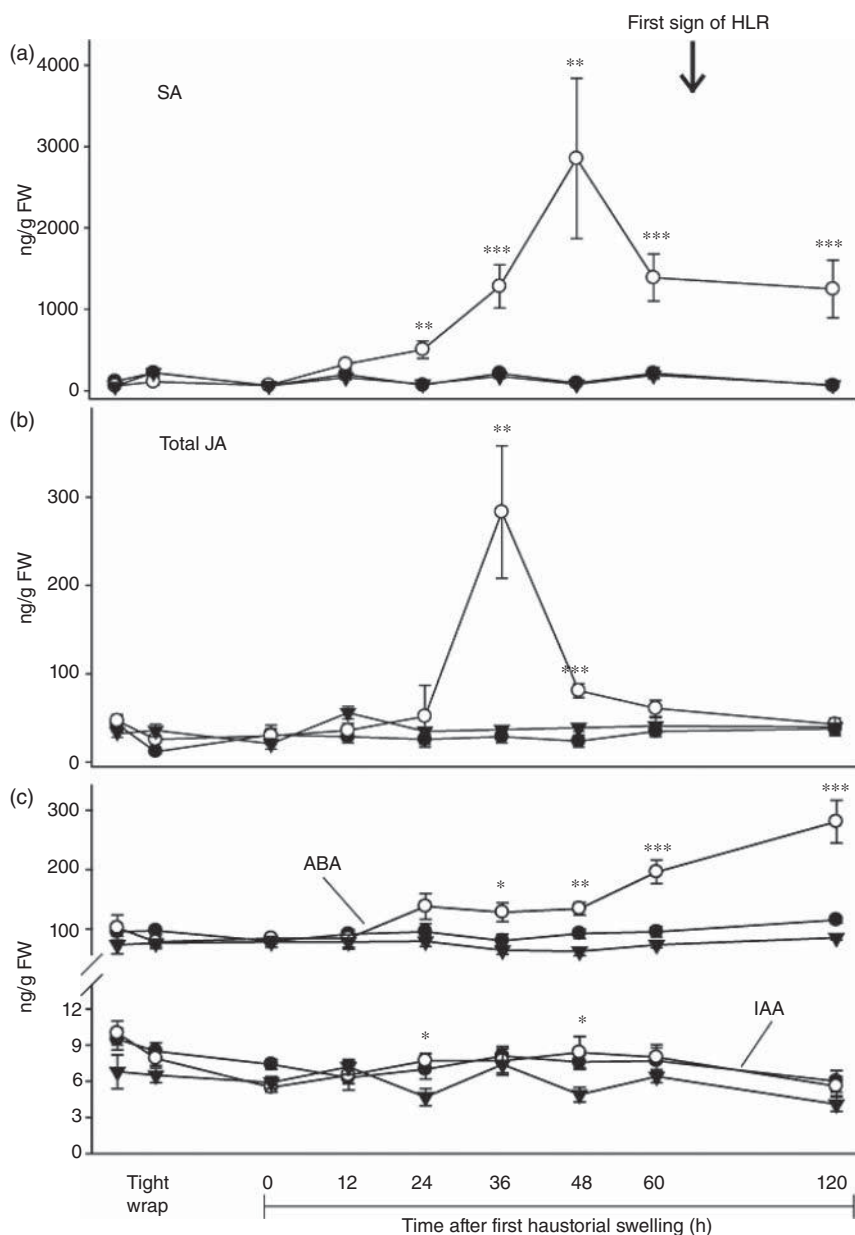


Fig. 8.12 Time course of changes in the phytohormones (a) salicylic acid (SA), (b) total jasmonic acid (JA), and (c) abscisic acid (ABA) and auxin [indole-3-acetic acid (IAA)] in 20-day-old tomato petioles during second attachment by *Cuscuta pentagona* seedlings. ABA and IAA are marked by lines and abbreviations. White circles represent parasitized petioles, dark circles represent unparasitized control petioles and dark triangles represent unparasitized petioles of plants with the first parasite attachment. Significant differences between treatments: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$. HLR, hypersensitive-like response. FW, fresh weight. Runyon *et al.* 2010. Reproduced with permission of John Wiley & Sons.

or auxin. Moreover, host signalling can be hijacked by the attacker, as a means of heading off full-scale defence deployment or helping to establish an effective sink for nutrients, or both. Hormones are important players in the battle between host and attacker, with the potential to determine the outcome of the interaction. As a result, understanding their roles in plant–attacker interactions could provide useful avenues for development of novel control measures.

RECOMMENDED READING

- Ashby AM, 2000. Biotrophy and the cytokinin conundrum. *Physiological and Molecular Plant Pathology* **57**, 147–158.
- Bari R, Jones JDG, 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69**, 473–488.
- Giron D, Frago E, Glevarec G, Pieterse CMJ, Dicke M, 2013. Cytokinins as key regulators in plant-microbe-insect interactions: connecting plant growth and defence. *Functional Ecology* **27**, 599–609.
- Kazan K, Manners JM, 2009. Linking development to defense: auxin in plant-pathogen interactions. *Trends in Plant Science* **14**, 373–382.
- Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM, 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28**, 489–521.
- Robert-Seilanianantz A, Grant M, Jones JD, 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* **49**, 317–343.
- Walters DR, McRoberts N, 2006. Plants and biotrophs: a pivotal role for cytokinins? *Trends in Plant Science* **11**, 581–586.

REFERENCES

- Achuo EA, Prinsen E, Hofte M, 2006. Influence of drought, salt stress and abscisic acid on the resistance of tomato to *Botrytis cinerea* and *Oidium neolycopersici*. *Plant Pathology* **55**, 178–186.
- Adie BAT, Pérez-Pérez J, Pérez-Pérez MM, Godoy M, Sánchez-Serrano J-J, Schmelz EA, Solano R, 2007. ABA is an essential signal for plant resistance to pathogens affecting JA biosynthesis and the activation of defences in *Arabidopsis*. *Plant Cell* **19**, 1665–1681.
- Albrecht C, Boutrot F, Segonzac C, Schwessinger B, Gimenez-Ibanez S, Chinchilla D, Rathjen JP, de Vries SC, Zipfel C, 2012. Brassinosteroids inhibit pathogen-associated molecular pattern-triggered immune signalling independent of the receptor kinase BAK1. *Proceedings of the National Academy of Sciences USA* **109**, 303–308.
- Alcázar R, Altabella T, Marco F, Bortolotti C, Reymond M, Koncz C, Carrasco P, Tiburcio AF, 2010. Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* **231**, 1237–1249.
- Ashby AM, 2000. Biotrophy and the cytokinin conundrum. *Physiological and Molecular Plant Pathology* **57**, 147–158.
- Asselbergh B, Achuo AE, Hofte M, Van Gijsegem F, 2008. Abscisic acid deficiency leads to rapid activation of tomato defence responses upon infection with *Erwinia chrysanthemi*. *Molecular Plant Pathology* **9**, 11–24.
- Audenaert K, De Meyer GB, Hofte MM, 2002. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signalling mechanisms. *Plant Physiology* **128**, 491–501.
- Bajguz A, 2007. Metabolism of brassinosteroids in plants. *Plant Physiology and Biochemistry* **45**, 95–107.
- Ballare CL, 2011. Jasmonate-induced defences: a tale of intelligence, collaborators and rascals. *Trends in Plant Science* **16**, 249–257.
- Bari R, Jones JDG, 2009. Role of plant hormones in plant defence responses. *Plant Molecular Biology* **69**, 473–488.
- Bar-Nun N, Sachs T, Mayer AM, 2008. A role for IAA in the infection of *Arabidopsis thaliana* by *Orobanchae aegyptiaca*. *Annals of Botany* **101**, 261–265.
- Barr KL, Hearne LB, Briesacher S, Clark TL, Davis GE, 2010. Microbial symbionts in insects influence down-regulation of defense genes in maize. *PLoS One* **5**, e11339.

- Belkhadir Y, Jaillais Y, Eppele P, Balsamão-Pires E, Dangel JL, Chory J, 2012. Brassinosteroids modulate the efficiency of plant immune responses to microbe-associated molecular patterns. *Proceedings of the National Academy of Sciences USA* **109**, 297–302.
- Bhardwaj V, Meier S, Petersen LN, Ingle RA, Roden LC, 2011. Defence responses of *Arabidopsis thaliana* to infection by *Pseudomonas syringae* are regulated by the circadian clock. *PLoS One* **6**, e26968.
- Blackman PG, Davies WJ, 1984. Modification of the CO₂ responses of maize stomata by abscisic acid and by naturally occurring and synthetic cytokinins. *Journal of Experimental Botany* **35**, 174–179.
- Bodenhausen N, Reymond P, 2007. Signaling pathways controlling induced resistance to insect herbivores in *Arabidopsis*. *Molecular Plant-Microbe Interactions* **20**, 1406–1420.
- Body M, Kaiser W, Dubreuil G, Casas J, Giron D, 2013. Leaf-miners co-opt microorganisms to enhance their nutritional environment. *Journal of Chemical Ecology* **39**, 969–977.
- Bostock RM, 1999. Signal conflicts and synergies in induced resistance to multiple attackers. *Physiological and Molecular Plant Pathology* **55**, 99–109.
- Bruce SA, Saville BJ, Emery RJN, 2011. *Ustilago maydis* produces cytokinins and abscisic acid for potential regulation of tumour formation in maize. *Journal of Plant Growth Regulation* **30**, 51–63.
- Bushnell WR, Allen PJ, 1962. Induction of disease symptoms in barley by powdery mildew. *Plant Physiology* **37**, 50–59.
- Chen Z, Agnew JL, Cohen JD, He P, Shan L, Sheen J, Kunkel BN, 2007. *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proceedings of the National Academy of Sciences USA* **104**, 20131–20136.
- Choi J, Huh SU, Kojima M, Sakakibara H, Paek K-H, Hwang I, 2010. The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signalling in *Arabidopsis*. *Developmental Cell* **19**, 284–295.
- Coghlan SE, Walters DR, 1990. Polyamine metabolism in ‘green islands’ on powdery mildew infected barley leaves: possible interactions with senescence. *New Phytologist* **116**, 417–424.
- Cooper SJ, Ashby AM, 1998. Comparison of cytokinin and cytokinin-O-glucoside cleaving beta-glucosidase production *in vitro* by *Venturia inaequalis* and other phytopathogenic fungi with differing modes of nutrition *in planta*. *Physiological and Molecular Plant Pathology* **53**, 61–72.
- Cowley T, Walters DR, 2002. Polyamine metabolism in barley reacting hypersensitively to the powdery mildew fungus *Blumeria graminis* f. sp. *hordei*. *Plant, Cell & Environment* **25**, 461–468.
- De Bock F, Fer A, 1992. Effects of abscisic acid on the transfer of sucrose from host, *Pelargonium zonale* (L.) Aiton, to a phanerogamic parasite, *Cuscuta reflexa* Roxb. *Australian Journal of Plant Physiology* **19**, 679–691.
- Dekhuijzen HM, 1981. The occurrence of free and bound cytokinins in plasmodia of *Plasmodiophora brassicae* isolated from tissue cultures of clubroots. *Plant Cell Reports* **1**, 18–20.
- Dekhuijzen HM, Overeem JC, 1971. The role of cytokinins in clubroot formation. *Physiological and Molecular Plant Pathology* **1**, 151–161.
- Dervinis C, Frost CJ, Lawrence SD, Novak NG, Davis JM, 2010. Cytokinin primes plant responses to wounding and reduces insect performance. *Journal of Plant Growth Regulation* **29**, 289–296.
- De Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Egea PR, Bogre L, Grant M, 2007. *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signalling pathway to cause disease. *EMBO Journal* **26**, 1434–1443.
- De Torres-Zabala M, Bennett MH, Truman WH, Grant MR, 2009. Antagonism between salicylic acid and abscisic acid reflects early host-pathogen conflict and moulds plant defence responses. *Plant Journal* **59**, 375–386.
- De Vos M, Van Oosten VR, Van Poecke RM, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Métraux J-P, Van Loon LC, Pieterse CMJ, 2005. Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. *Molecular Plant-Microbe Interactions* **18**, 923–937.
- Devos S, Laukens K, Deckers P, Van der Straeten D, Beeckman T, Inze D, Van Onckelen H, Witters E, Prinsen E, 2006. A hormone and proteome approach to picturing the initial metabolic events during *Plasmodiophora brassicae* infection on *Arabidopsis*. *Molecular Plant-Microbe Interactions* **19**, 1431–1443.
- Devos S, Vissenberg K, Verbelen J-P, Prinsen E, 2005. Infection of Chinese cabbage by *Plasmodiophora brassicae* leads to a stimulation of plant growth: impacts on cell wall metabolism and hormone balance. *New Phytologist* **166**, 241–250.
- Dinh ST, Baldwin IT, Galis I, 2013. The *HERBIVORE ELICITOR-REGULATED1* gene enhances abscisic acid levels and defences against herbivores in *Nicotiana attenuata* plants. *Plant Physiology* **162**, 2106–2124.

- Dörr I, 1996. New results on interspecific bridges between parasites and their hosts. In: Moreno T, Cubero JI, eds. *Advances in parasitic plant research*. Congreos y Jornadas, 36/96, Junta de Andalucía, Consejería de Agricultura y Pesca, pp. 196–201.
- Dörr I, Kollman R, 1995. Symplastic sieve element continuity between *Orobanch*e and its host. *Botanica Acta* **108**, 47–55.
- Drennan DSH, El Hiweris SO, 1979. Changes in growth regulating substances in *Sorghum vulgare* infected by *Striga hermonthica*. In: Musselman LJ, Worsham AD, Eplee RE, eds. *Proceedings of the second symposium of parasitic weeds*. North Carolina State University, Raleigh, NC, USA, pp. 144–155.
- Elzen GW, 1983. Cytokinins and insect galls. *Comparative Biochemistry and Physiology A*, **76**, 17–19.
- Engelbrecht L, Orban U, Heese W, 1969. Leaf-miner caterpillars and cytokinins in the green islands of autumn leaves. *Nature* **223**, 319–321.
- Engelbrecht L, 1971. Cytokinin activity in larval infected leaves. *Biochemie und Physiologie der Pflanzen* **162**, 9–27.
- Erb M, Flors V, Karlen D, de Lange E, Planchamp C, D'Alessandro M, Turlings TCJ, Ton J, 2009. Signal signature of aboveground-induced resistance upon belowground herbivory in maize. *The Plant Journal* **59**, 292–302.
- Erb M, Köllner TG, Degenhardt J, Zwahlen C, Hibbard BE, Turlings TCJ, 2011. The role of abscisic acid and water stress in root herbivore-induced leaf resistance. *New Phytologist* **189**, 308–320.
- Erb M, Meldau S, Howe GA, 2012. Role of phytohormones in insect-specific plant reactions. *Trends in Plant Science* **17**, 250–259.
- Evangelisti E, Govetto B, Minet-Kebdani N, Kuhn M-L, Attard A, Ponchet M, Panabières F, Gourgues M, 2013. The *Phytophthora parasitica* RXLR effector Penetration-Specific Effector 1 favours *Arabidopsis thaliana* infection by interfering with auxin physiology. *New Phytologist* **199**, 476–489.
- Fan J, Hill L, Crooks C, Doerner P, Lamb C, 2009. Absciscic acid has a key role in modulating diverse plant-pathogen interactions. *Plant Physiology* **150**, 1750–1761.
- Flors V, Ton J, Van Doorn R, Jakab G, Garcia-Agustin P, Mauch-Mani P, 2008. Interplay between JA, SA and ABA signalling during basal and induced resistance against *Pseudomonas syringae* and *Alternaria brassicicola*. *Plant Journal* **54**, 81–92.
- Foster SA, Walters DR, 1992. Polyamine concentrations and activities of ornithine and arginine decarboxylase in wheat infected with the stem rust fungus. *Journal of Plant Physiology* **140**, 134–136.
- Frost DL, Gurney AL, Press MC, Scholes JD, 1997. *Striga hermonthica* reduces photosynthesis in sorghum: the importance of stomatal limitations and a potential role for ABA? *Plant, Cell and Environment* **20**, 483–492.
- Gilardoni PA, Schuck S, Jungling R, Rotter B, Baldwin IT, Bonaventure G, 2010. SuperSAGE analysis of the *Nicotiana attenuata* transcriptome after fatty acid amino acid elicitation (FAC): identification of early mediators of insect responses. *BMC Plant Biology* **10**, 66.
- Giron D, Kaiser W, Imbault N, Casas J, 2007. Cytokinin-mediated leaf manipulation by a leafminer caterpillar. *Biology Letters* **3**, 340–343.
- Giron D, Frago E, Glevarec G, Pieterse CMJ, Dicke M, 2013. Cytokinins as key regulators in plant-microbe-insect interactions: connecting plant growth and defence. *Functional Ecology* **27**, 599–609.
- Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pages V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Becard G, Beveridge CA, Rameau C, Rochange SF, 2008. Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194.
- Gonzalez ME, Marco F, Minguet EG, Carrasco-Sorli P, Blázquez MA, Carbonell J, Ruiz OA, Pieckenstein FL, 2011. Perturbation of *spermine synthase* gene expression and transcript profiling provide new insights on the role of the tetraamine spermine in *Arabidopsis* defense against *Pseudomonas viridiflava*. *Plant Physiology* **156**, 2266–2277.
- Goodspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF, 2012. *Arabidopsis* synchronizes jasmonate-mediated defense with insect circadian behaviour. *Proceedings of the National Academy of Sciences USA* **109**, 4674–4677.
- Goverse A, Overmars H, Engelbertink J, Schots A, Bakker J, Helder J, 2000. Both induction and morphogenesis of cyst nematode feeding cells are mediated by auxin. *Molecular Plant-Microbe Interactions* **13**, 1121–1129.
- Graf A, Schlereth A, Stitt M, Smith AM, 2010. Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. *Proceedings of the National Academy of Sciences USA* **107**, 9458–9463.
- Greenland A, Lewis DH, 1984. Amines in barley leaves infected with brown rust and their possible relevance to formation of 'green islands'. *New Phytologist* **96**, 283–291.

- Großkinsky DK, Naseem M, Abdelhohsen UR, Plickert N, Engelke T, Griebel T, Zeier J, Novák O, Strnad M, Pfeifhofer H, van der Graaff E, Simon U, Roitsch T, 2011. Cytokinins mediate resistance against *Pseudomonas syringae* in tobacco through increased antimicrobial phytoalexin synthesis independent of salicylic acid signalling. *Plant Physiology* **157**, 815–830.
- Grsic-Rausch S, Kobelt P, Siemens J, Bischoff M, Ludwig-Müller J, 2000. Expression and localization of nitrilase during symptom development of the clubroot disease in *Arabidopsis thaliana*. *Plant Physiology* **122**, 369–378.
- Grunewald W, Karimi M, Wieczorek K, Van de Capelle E, Wischnitzki E, Grundler F, Inzé D, Beeckman T, Gheysen G, 2008. A role for AtWRKY23 in feeding site establishment of plant-parasitic nematodes. *Plant Physiology* **148**, 358–368.
- Grunewald W, Canoot B, Friml J, Gheysen G, 2009. Parasitic nematodes modulate PIN-mediated auxin transport to facilitate infection. *PLoS Pathogens* **5**, e1000266.
- Hanano S, Domagalska MA, Nagy F, Davis SJ, 2006. Multiple phytohormones influence distinct parameters of the plant circadian clock. *Genes to Cells* **11**, 1381–1392.
- Hodson MJ, Bryant JA, 2012. *Functional biology of plants*. Oxford: Wiley-Blackwell.
- Hori K, 1992. Insect secretions and their effect on plant growth, with special reference to Hemipterans. In: Shorthouse JD, Rohfritsch O, eds. *Biology of insect-induced plant galls*. New York: Oxford University Press, pp. 157–170.
- Hui DQ, Iqbal J, Lehmann K, Gase K, Saluz HP, Baldwin IT, 2003. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterisation of large-scale changes in herbivore-induced mRNAs. *Plant Physiology* **131**, 1877–1893.
- Jiang C-J, Shimono M, Sugano S, Kojima M, Liu X, Inoue H, Sakakibara H, Takatsuji H, 2013. Cytokinins act synergistically with salicylic acid to activate defense gene expression in rice. *Molecular Plant-Microbe Interactions* **26**, 287–296.
- Jiang F, Jeschke WD, Hartung W, 2004. Abscissic acid (ABA) flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite abscissic acid relations. *Journal of Experimental Botany* **55**, 2323–2329.
- Jiang F, Veselova S, Veselov D, Kudoyarova G, Jeschke WD, Hartung W, 2005. Cytokinin flows from *Hordeum vulgare* to the hemiparasite *Rhinanthus minor* and the influence of infection on host and parasite cytokinin relations. *Functional Plant Biology* **32**, 619–629.
- Kaiser W, Huguet E, Casas J, Commin C, Giron D, 2010. Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society of London, series B* **227**, 2311–2319.
- Karczmarek A, Overmars H, Helder J, Goverse A, 2004. Feeding cell development by cyst and root-knot nematodes involves a similar early, local and transient activation of a specific auxin-inducible promoter element. *Molecular Plant Pathology* **5**, 343–346.
- Kazan K, Manners JM, 2009. Linking development to defense: auxin in plant-pathogen interactions. *Trends in Plant Science* **14**, 373–382.
- Kemmerling B, Schwedt A, Rodriguez P, Mazzota S, Frank M, Abu Qamar S, Mengiste T, Betsuyaku S, Parker JE, Mussig C, Thomma BPHJ, Albrecht C, de Vries SC, Hirt H, Nurnberger T, 2007. The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Current Biology* **17**, 1116–1122.
- Kempema LA, Cui X, Holzer FM, Walling LL, 2007. *Arabidopsis* transcriptome changes in response to phloem-feeding silverleaf whitefly nymphs. Similarities and distinctions in responses to aphids. *Plant Physiology* **143**, 849–865.
- Kidd BN, Kadoo NY, Dombrecht B, Tekeoglu M, Gardiner DM, Thatcher LF, Aitken EAB, Schenk PM, Manners JM, Kazan K, 2011. Auxin signalling and transport promote susceptibility to the root-infecting fungal pathogen *Fusarium oxysporum* in *Arabidopsis*. *Molecular Plant-Microbe Interactions* **24**, 733–748.
- Kiraly Z, Pozsar BI, El Hammady M, 1966. Cytokinin activity of rust-infected plants: juvenility and senescence in diseased leaf tissues. *Acta Phytopathological Academy of Science Hungary* **1**, 29–37.
- Kiraly Z, El Hammady M, Pozsar BI, 1967. Increased cytokinin activity of rust-infected bean and broad bean leaves. *Phytopathology* **57**, 93–94.
- Kobelt P, Siemens J, Sacristan MD, 2000. Histological characterisation of the incompatible interaction between *Arabidopsis thaliana* and the obligate biotrophic pathogen *Plasmodiophora brassicae*. *Mycological Research* **104**, 220–225.

- Lee C, Chronis D, Kenning C, Peret B, Hewezi T, Davis EL, Baum TJ, Hussey R, Bennett M, Mitchum MG, 2011. The novel cyst nematode effector protein 19C07 interacts with the *Arabidopsis* auxin influx transporter LAX3 to control feeding site development. *Plant Physiology* **155**, 866–880.
- Li Q, Xie QG, Smith-Becker J, Navarre DA, Kaloshian I, 2006. Mi-1-mediated aphid resistance involves salicylic acid and mitogen-activated protein kinase signalling cascades. *Molecular Plant-Microbe Interactions* **19**, 655–664.
- Llorente F, Muskett P, Sánchez-Vallet A, López G, Ramos B, Sánchez-Rodríguez C, Jordá L, Parker J, Molina A, 2008. Repression of the auxin response pathway increases *Arabidopsis* susceptibility to necrotrophic fungi. *Molecular Plant* **1**, 496–509.
- Lopez-Carbonell M, Moret A, Nadal M, 1998. Changes in cell ultrastructure and zeatin riboside concentrations in *Hedera helix*, *Pelargonium zonale*, *Prunus avium*, and *Rubus ulmifolius* leaves infected by fungi. *Plant Disease* **82**, 914–918.
- Lu SX, Knowles SM, Andronis C, Ong MS, Tobin EM, 2009. *CIRCADIAN CLOCK ASSOCIATED 1* and *LATE ELONGATED HYPOCOTYL* function synergistically in the circadian clock of *Arabidopsis*. *Plant Physiology* **150**, 834–843.
- Ludwig-Müller J, Epstein E, Hilgengerg W, 1996. Auxin-conjugate hydrolysis in Chinese cabbage: characterization of an amidohydrolase and its role during the clubroot disease. *Physiologia Plantarum* **97**, 627–634.
- Ludwig-Müller J, Pieper K, Ruppel M, Cohen JD, Epstein E, Kiddle G, Bennett R, 1999. Indole glucosinolate and auxin biosynthesis in *Arabidopsis thaliana* L. glucosinolate mutants and development of the clubroot disease. *Planta* **208**, 409–419.
- Ludwig-Müller J, Prinsen E, Rolfe SA, Scholes JD, 2009. Metabolism and plant hormone action during clubroot disease. *Journal of Plant Growth Regulation* **28**, 229–244.
- Machado RAR, Ferrieri AP, Robert CAM, Glauser G, Kallenbach M, Baldwin IT, Erb M, 2013. Leaf-herbivore attack reduces carbon reserves and regrowth from the roots via jasmonate and auxin signalling. *New Phytologist* **200**, 1234–1246.
- Malinowski R, Smith JA, Fleming AJ, Scholes JD, Rolfe SA, 2012. Gall formation in clubroot-infected *Arabidopsis* results from an increase in existing meristematic activities of the host but is not essential for the completion of the pathogen life cycle. *The Plant Journal* **71**, 226–238.
- Mansfield TA, McAinsh MR, 1995. Hormones as regulators of water balance. In: Davies PJ, ed. *Plant hormones – physiology, biochemistry and molecular biology*. Dordrecht: Kluwer Academic Publishers, pp. 598–616.
- Mapes CC, Davies PJ, 2001a. Indole-3-acetic acid and ball gall development on *Solidago altissima*. *New Phytologist* **151**, 195–202.
- Mapes CC, Davies PJ, 2001b. Cytokinins in the ball gall of *Solidago altissima* and in the gall forming larvae of *Eurosta solidaginis*. *New Phytologist* **151**, 203–212.
- Martinez S, Cordova I, Maust BE, Oropeza C, Santamaria JM, 2000. Is abscisic acid responsible for abnormal stomatal closure in coconut palms showing lethal yellowing? *Journal of Plant Physiology* **156**, 319–322.
- Mazarei M, Lennon KA, Puthoff DP, Rodermel SR, Baum TJ, 2003. Expression of an *Arabidopsis* phosphoglycerate mutase homologue is localized to apical meristems, regulated by hormones, and induced by sedentary plant-parasitic nematodes. *Plant Molecular Biology* **53**, 513–530.
- Michael TP, Salome PA, Yu HJ, Spencer TR, Sharp EL, McPeck MA, Alonso JM, Ecker JR, McClung CR, 2003. Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* **302**, 1049–1053.
- Mills LJ, Van Staden J, 1978. Extraction of cytokinins from maize, smut tumours of maize and *Ustilago maydis* cultures. *Physiological Plant Pathology* **13**, 73–80.
- Moschou PN, Sarris PF, Skandalis N, Andriopoulou AH, Paschalidis KA, Panopoulos NJ, Roubelakis-Angelakis KA, 2009. Engineered polyamine catabolism preinduces tolerance of tobacco to bacteria and oomycetes. *Plant Physiology* **149**, 1970–1981.
- Moulton JE, 1942. Extraction of auxin from maize, smut tumours of maize, and from *Ustilago zeae*. *Botanical Gazette* **103**, 725–739.
- Müller P, Hilgengerg W, 1986. Isomers of zeatin and zeatin riboside in clubroot disease: evidence for trans-zeatin biosynthesis by *Plasmodiophora brassicae*. *Physiologia Plantarum* **66**, 245–250.
- Müller B, Sheen J, 2007. Advances in cytokinin signalling. *Science* **318**, 68–69.
- Murphy AM, Pryce-Jones E, Johnstone K, Ashby AM, 1997. Comparison of cytokinin production *in vitro* by *Pyrenopeziza brassicae* with other plant pathogens. *Physiological and Molecular Plant Pathology* **50**, 53–65.

- Mutka AM, Fawley S, Tsao T, Kunkel BN, 2013. Auxin promotes susceptibility to *Pseudomonas syringae* via a mechanism independent of suppression of salicylic acid-mediated defences. *The Plant Journal* **74**, 746–754.
- Nambeesan S, AbuQamar S, Laluk K, Mattoo AK, Micklebart MV, Ferruzzi MG, Mengiste T, Handa AK, 2012. Polyamines attenuate ethylene-mediated defense responses to abrogate resistance to *Botrytis cinerea* in tomato. *Plant Physiology* **158**, 1034–1045.
- Naseem M, Dandekar T, 2012. The role of auxin-cytokinin antagonism in plant-pathogen interactions. *PLoS Pathogens* **8**(11), e1003026.
- Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JD, 2006. A plant miRNA contributes to antibacterial resistance by repressing auxin signalling. *Science* **312**, 436–439.
- Navarro L, Bari R, Achard P, Lison P, Nemri A, Harberd NP, Jones JD, 2008. DELLAs control plant immune responses by modulating the balance of jasmonic acid and salicylic acid signalling. *Current Biology* **18**, 650–655.
- O'Donnell PJ, Schemlitz EA, Moussatche P, Lund ST, Jones JB, Klee HJ, 2003. Susceptible to intolerance – a range of hormonal actions in a susceptible *Arabidopsis* pathogen response. *Plant Journal* **33**, 245–257.
- Onkokesung N, Galis I, von Dahl CC, Matsuoka K, Saluz HP, Baldwin IT, 2010. Jasmonic acid and ethylene modulate local responses to wounding and simulated herbivory in *Nicotiana attenuata* leaves. *Plant Physiology* **153**, 785–798.
- Pauwels L, Inze D, Goossens A, 2009. Jasmonate-inducible gene: what does it mean? *Trends in Plant Science* **14**, 87–91.
- Pegg GF, 1981. The involvement of growth regulators in the diseased plant. In: Ayres PG, ed. *Effects of disease on the physiology of the growing plant*. Cambridge: Cambridge University Press, pp. 149–178.
- Pieterse CMJ, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SCM, 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28**, 489–521.
- Qi L, Yan J, Li Y, Jiang H, Sun J, Chen Q, Li H, Chu J, Yan C, Sun X, Yu Y, Li C, Li C, 2012. *Arabidopsis thaliana* plants differentially modulate auxin biosynthesis and transport during defense responses to the necrotrophic pathogen *Alternaria brassicicola*. *New Phytologist* **195**, 872–882.
- Rawat R, Schwatrz J, Jones MA, Sairanen I, Cheng YF, Andersson CR, Zhao YD, Ljung K, Harmer SL, 2009. REVEILLE1, a Myb-like transcription factor, integrates the circadian clock and auxin pathways. *Proceedings of the National Academy of Sciences USA* **106**, 16883–16888.
- Reineke G, Heinze B, Schirawski J, Buettner H, Kahmann R, Basse CW, 2008. Indole-3-acetic acid (IAA) biosynthesis in the smut fungus *Ustilago maydis* and its relevance for increased IAA levels in infected tissue and host tumour formation. *Molecular Plant Pathology* **9**, 339–355.
- Reinhardt D, Pesce ER, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlmeier C, 2003. Regulation of phyllotaxis by polar auxin transport. *Nature* **426**, 255–260.
- Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE, 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell* **16**, 3132–3147.
- Roberts AM, 1987. Photosynthesis, nitrogen metabolism and shoot:root equilibria in leeks infected with the rust *Puccinia allii*. *PhD Thesis, University of Glasgow, UK*.
- Robert-Seilaniantz A, Grant M, Jones JD, 2011. Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. *Annual Review of Phytopathology* **49**, 317–343.
- Robertson FC, Skeffington AW, Gardner MJ, Webb AA, 2009. Interactions between circadian and hormonal signalling in plants. *Plant Molecular Biology* **69**, 419–427.
- Roden LC, Ingle RA, 2009. Lights, rhythms, infection: the role of light and the circadian clock in determining the outcome of plant-pathogen interactions. *Plant Cell* **21**, 2546–2552.
- Runyon JB, Mescher MC, Felton GW, De Moraes CM, 2010. Parasitism by *Cuscuta pentagona* sequentially induces JA and SA defence pathways in tomato. *Plant, Cell and Environment* **33**, 290–303.
- Sachs T, 1981. The control of patterned differentiation of vascular tissues. *Advances in Botanical Research* **9**, 151–162.
- Sano H, Seo S, Koizumi N, Niki T, Iwamura H, Ohashi Y, 1996. Regulation by cytokinins of endogenous levels of jasmonic and salicylic acids in mechanically wounded tobacco plants. *Plant and Cell Physiology* **37**, 762–769.
- Scarpella E, Marcos D, Friml J, Berleth T, 2006. Control of leaf vascular patterning by polar auxin transport. *Genes and Development* **20**, 2015–2027.
- Siemens J, Keller I, Sarx J, Kunz S, Schuller A, Nagel W, Schmülling T, Parniske M, Ludwig-Müller J, 2006. Transcriptome analysis of *Arabidopsis* clubroots indicate a key role for cytokinins in disease development. *Molecular Plant-Microbe Interactions* **19**, 480–494.

- Smigocki A, Neal JW, McCanna I, Douglass L, 1993. Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the IPT gene. *Plant Molecular Biology* **23**, 325–335.
- Smith AM, Coupland G, Dolan L, Harberd N, Jones J, Martin C, Sablowski R, Amey A, 2010. *Plant biology*. New York: Garland Science.
- Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W, 2005. Characterisation of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. *Plant Cell* **17**, 616–627.
- Suwa H, Suzuki Y, Zhang Y-H, Murofushi N, Takeuchi Y, 1995. Endogenous gibberellins in *Aeginetia indica*, a parasitic plant, and its host, *Miscanthus sinensis*. *Bioscience, Biotechnology and Biochemistry* **59**, 1712–1715.
- Suzuki Y, Zhang Y-H, Murofushi N, Takeuchi Y, 1994. Endogenous gibberellins in clover broomrape, a parasitic plants, and its host, clover – dependency of the parasite on the host for gibberellin production. *Journal of Plant Growth Regulation* **13**, 63–67.
- Takahashi T, Takechi J-I, 2010. Polyamines: ubiquitous polycations with unique roles in growth and stress responses. *Annals of Botany* **105**, 1–6.
- Tanaka N, Matsuoka M, Kitano H, Asano T, Kaku H, Komatsu S, 2006. *gid1*, a gibberellin-insensitive mutant, shows altered regulation of probenazole-inducible protein (PBZ1) in response to cold stress and pathogen attack. *Plant, Cell and Environment* **29**, 619–631.
- Taylor A, Martin J, Seel WE, 1996. Physiology of the parasitic association between maize and witchweed (*Striga hermonthica*): is ABA involved? *Journal of Experimental Botany* **47**, 1057–1065.
- Thain SC, Vandenbussche F, Laarhoven LJ, Dowson-Day MJ, Wang ZY, Tobin EM, Harren FJM, Millar AJ, Van der Straeten D, 2004. Circadian rhythms of ethylene emission in *Arabidopsis*. *Plant Physiology* **136**, 3751–3761.
- Thaler JS, Bostock RM, 2004. Interactions between abscisic acid-mediated responses and plant resistance to pathogens and insects. *Ecology* **85**, 48–58.
- Tomilov AA, Tomilova N, Yoder JJ, 2004. *In vitro* haustorium formation in roots and root cultures of the hemiparasitic plant *Triphysaria versicolor*. *Plant, Cell and Organ Culture* **77**, 257–265.
- Tomilov AA, Tomilova N, Addallah I, Yoder JJ, 2005. Localized hormone fluxes and early haustorium development in the hemiparasitic plant *Triphysaria versicolor*. *Plant Physiology* **138**, 1469–1480.
- Tooker JF, De Moraes CM, 2005. Jasmonate in lepidopteran eggs and neonates. *Journal of Chemical Ecology* **31**, 2753–2759.
- Tooker JF, De Moraes CM, 2011. Feeding by a gall-inducing caterpillar species alters levels of indole-3-acetic and abscisic acid in *Solidago altissima* (Asteraceae) stems. *Arthropod Plant Interactions* **5**, 115–124.
- Torrigiani P, Rabiti AL, Bortolotti G, Betti L, Marani F, Canova A, Bagni N, 1997. Polyamine synthesis and accumulation in the hypersensitive response to TMV in *Nicotiana tabacum*. *New Phytologist* **135**, 467–473.
- Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, Takeda-Kamiya N, Magome H, Kamiya Y, Shirasu K, Yoneyama K, Kyoizuka J, Yamaguchi S, 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195–200.
- Walters DR, 2003a. Polyamines and plant disease. *Phytochemistry* **64**, 97–107.
- Walters DR, 2003b. Resistance to plant pathogens: possible roles for free polyamines and polyamine catabolism. *New Phytologist* **159**, 109–115.
- Walters DR, Wilson PWF, Shuttleton MA, 1985. Relative changes in levels of polyamines and activities of biosynthetic enzymes in barley infected with the powdery mildew fungus, *Erysiphe graminis* DC. ex Merat f.sp. *hordei* Marshal. *New Phytologist* **101**, 695–705.
- Walters DR, Wyllie MA, 1986. Polyamines in discrete regions of barley leaves infected with the powdery mildew fungus *Erysiphe graminis*. *Physiologia Plantarum* **67**, 630–633.
- Walters DR, McRoberts N, 2006. Plants and biotrophs: a pivotal role for cytokinins? *Trends in Plant Science* **11**, 581–586.
- Walters DR, McRoberts N, Fitt BDL, 2008. Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biological Reviews* **83**, 79–102.
- Walters DR, 2010. *Plant defense: warding off attack by pathogens, herbivores and parasitic plants*. Oxford: Wiley-Blackwell.
- Wang D, Pajeroska-Mukhtar K, Culler AH, Dong X, 2007a. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signalling pathway. *Current Biology* **17**, 1784–1790.
- Wang L, Halitschke R, Kang JH, Berg A, Harnisch F, Baldwin IT, 2007b. Independently silencing two JAR family members impairs levels of trypsin proteinase inhibitors but not nicotine. *Planta* **226**, 159–167.

- Wang W, Barnaby JY, Tada Y, Li H, Tor M, Caldelari D, Lee DU, Fu XD, Dong XN, 2011. Timing of plant immune responses by a central circadian regulator. *Nature* **470**, 110–114.
- Wasilewskaa A, Vlad F, Sirichandra C, Redko Y, Jammes F, Valon C, Frey NFD, Leung J, 2008. An update on abscisic acid signalling in plants and more. *Molecular Plant* **1**, 198–217.
- Weyman PD, Pan Z, Feng Q, Gilchrist DG, Bostock RM, 2006. A circadian rhythm-regulated tomato gene is induced by arachidonic acid and *Phytophthora infestans* infection. *Plant Physiology* **140**, 235–248.
- Woodward AW, Bartel B, 2005. Auxin: regulation, action, and interaction. *Annals of Botany* **95**, 707–735.
- Xing Y, Jia W, Zhang J, 2008. AtMKK1 mediates ABA-induced CAT1 expression and H₂O₂ production via AtMPK6-coupled signalling in *Arabidopsis*. *Plant Journal* **54**, 440–451.
- Yamada T, 1993. The role of auxin in plant-disease development. *Annual Review of Phytopathology* **31**, 253–273.
- Yamakawa H, Kamada H, Satoh M, Ohashi Y. 1998. Spermine is a salicylate-independent endogenous inducer for both tobacco acidic pathogenesis-related proteins and resistance against tobacco mosaic virus infection. *Plant Physiology* **118**, 1213–1222.
- Yang D-L, Li Q, Deng Y-W, Lou Y-G, Wang M-Y, Zhou G-X, Zhang Y-Y, He Z-H, 2008. Altered disease development in the *eui* mutants and *eui* overexpressors indicates that gibberellins negatively regulate rice basal disease resistance. *Molecular Plant* **1**, 528–537.
- Zhang C, Xie Q, Anderson RG, Ng G, Seitz NC, Peterson T, McClung CR, McDowell JM, Kong D, Kwak JM, Lu H, 2013. Crosstalk between the circadian clock and innate immunity in *Arabidopsis*. *PLoS Pathogens* **9**, e1003370.

9 Bringing It Together: Physiology and Metabolism of the Attacked Plant

9.1 INTRODUCTION

We have spent the last eight chapters looking at the changes that occur in the physiology and metabolism of plants under attack. We have examined these changes individually, separating changes in photosynthesis, from those in carbohydrate metabolism, nutrient relations and growth regulators. However, many of the changes in physiology and metabolism are connected and in resistant hosts, for example, might represent trade-offs between defence and primary metabolism, whereas in susceptible hosts, these changes might represent the plant's attempt to minimise the damage being inflicted by the attacker. The advent of genomics, proteomics and metabolomics has afforded us the opportunity to follow these changes at a level of detail that was not possible 30 years ago. More importantly, it has allowed us to begin to piece together the sequence of events in the interaction between a plant and its attacker and to determine how the changes in metabolism and physiology are linked and how they relate to the final outcome of the interaction. Some recent large studies have attempted to do just that and will provide the focus for this chapter.

9.2 METABOLIC REPROGRAMMING IN PLANT–PATHOGEN INTERACTIONS

The smut fungus *Ustilago maydis* is a biotroph that induces tumours on aerial parts of its maize host. During the early stages of infection, invading hyphae are surrounded by the host plasma membrane, and during later stages in the interaction, hyphal growth is both intracellular and intercellular. Large plant tumours develop, associated with both enlargement of plant cells and increased cell division, and fungal proliferation occurs within these tumours. Using a combination of confocal microscopy, global expression profiling and metabolic profiling, Doehlemann *et al.* (2008) found that although the pathogen is recognised by the plant early and defence responses are triggered, with the onset of biotrophy 24 hours after inoculation, defences are down-regulated. Indeed, PR1 expression was undetectable early in the interaction and was only

expressed at a low level later in the interaction. Interestingly, induction of jasmonic acid (JA) signalling was detected immediately after infection, and there was transcriptional activation of both auxin synthesis and auxin-responsive genes during tumour development. Since induction of JA signalling antagonises salicylic acid (SA) signalling (Glazebrook, 2005), and SA is known to repress auxin signalling in *Arabidopsis* (Wang *et al.*, 2007), the authors suggested that in the *U. maydis*/maize interaction, JA inhibition of SA signalling allows auxin signalling to occur, which, in turn, promotes fungal growth and susceptibility (Doehlemann *et al.*, 2008).

Although there was a global induction of genes involved in the light reactions of photosynthesis, the Calvin cycle, photorespiration, tetrapyrrole synthesis and the synthesis of sucrose and starch in uninfected maize leaves, no such induction was observed in infected leaves. It appeared therefore that the transition from juvenile sink tissue to mature, photosynthetically active source tissue was blocked in infected leaves, a finding in line with reports that maize leaves infected by *U. maydis* are unable to establish C₄ photosynthesis, but continue C₃ photosynthesis, as usually observed in immature maize leaves (Horst *et al.*, 2008). Furthermore, infected leaves also exhibit reduced photosynthetic rates, coupled with reduced chlorophyll contents and pronounced chlorosis (Horst *et al.*, 2008). In fact, some 60% of the differentially expressed genes in *U. maydis*-infected leaves are attributable to down-regulation of the photosynthetic apparatus (Doehlemann *et al.*, 2008). Glutathione content was increased in infected leaves throughout the interaction, probably reflecting the need for an enhanced antioxidative capacity after the disruption of the photosynthetic machinery of the leaf.

Magnaporthe grisea, the causal agent of rice blast, is traditionally thought of as a hemibiotrophic pathogen. Using hydrostatic turgor, the fungus pushes its penetration peg through the host cuticle, entering epidermal cells about 24 hours after fungal spores have landed on the leaf surface (Talbot, 2003). In a susceptible host plant, a primary hypha is produced from the infection peg, followed by invasive hyphae that can fill the compromised host cell within 48 hours. Importantly, the fungal hyphae are ensheathed by the host plasma membrane, and the initial host cell remains intact. Death of host cells only occurs after extensive colonisation of host tissue by the fungus, with chlorosis of host tissue and lesion formation occurring approximately 72–96 hours after initial fungal penetration.

In a resistant host, invasion by *M. grisea* is usually halted within 48 hours as a result of localised defences, including production of reactive oxygen species (ROS), cell wall reinforcement and induction of a hypersensitive response (HR) (Talbot, 2003). In contrast, in susceptible hosts, such rapid defence activation appears to be suppressed. Parker *et al.* (2009) used metabolite fingerprinting and metabolite profiling to study the interaction of *M. grisea* on three susceptible hosts: rice, barley and *Brachypodium distachyon*. They found that the pathogen effected a sophisticated reprogramming of host metabolism in pre-symptomatic tissues, when the fungus was still in the process of penetrating individual epidermal cells. Thus, malate and polyamines accumulated, instead of being used to generate ROS for defence, while levels of metabolites associated with amelioration of oxidative stress increased considerably. In susceptible hosts, the pathogen modulated phenylpropanoid metabolism, rendering the host incapable of mounting a HR or producing lignified papillae to restrict pathogen invasion. After 3 days, there was rapid fungal growth in host tissue, associated with greatly increased nutrient acquisition and utilisation by the pathogen. Interestingly, by 48 hours, there were large increases in sucrose and aspartate, major sources of carbon and nitrogen, respectively, both of which are transported long distances within the plant. Since at 48 hours, fungal hyphae are still largely confined to invaded epidermal cells, it would appear that *M. grisea* is able

to modulate metabolism and metabolite transport in distant host tissues, while still largely confined to epidermal cells (Parker *et al.*, 2009).

Magnaporthe grisea grew biotrophically as it colonised host tissue, with symptom development occurring between 72 and 96 hours after infection. Formation of necrotic lesions appeared to result from de-repression of host defences, which were actively suppressed during the early stages of the interaction (Parker *et al.*, 2009). No evidence could be found for deployment of toxins by *M. grisea* to kill host cells during symptom development, although Parker *et al.* (2009) thought it likely that fungal sporulation 5–6 days after infection was fuelled by nutrient release from dying cells.

Similarly to other successful bacterial plant pathogens, *Pseudomonas syringae* pv. *tomato* (*Pst*) strain DC3000 has evolved a collection of effector proteins, delivered by the type III secretion system (T3SS), which suppress the basal defences of the host. *Pst* DC3000 populations can be greater than 5×10^7 colony-forming units per square centimetre of leaf in a compatible interaction, and in order to sustain such a large bacterial population, the type three effectors (TTEs) of the bacteria must reprogram host metabolism in order to provide sufficient nutrient resources. This was examined by Ward *et al.* (2010) in *Arabidopsis thaliana* using the virulent *Pst* DC3000 and a T3SS-compromised strain of *Pst* DC3000 (*DC3000 hrpA*). They demonstrated clear differences in the metabolome of *Arabidopsis* plants infected with *Pst* DC3000 within 8 hours of infection, including rapid alterations in the abundance of amino acids and other nitrogenous compounds, disaccharides and molecules that influence the accumulation of ROS. This study highlighted a rapid, coordinated reconfiguration of host metabolism in order to suppress defence responses and provide the nutrient resources required to support a rapidly growing bacterial population.

In an attempt to determine whether common motifs could be revealed in the response of primary carbon and nitrogen metabolism towards colonisation with biotrophic fungal pathogens in cereals, Voll *et al.* (2011) conducted a combined metabolome and transcriptome study of three different pathosystems: (i) barley/powdery mildew (*Blumeria graminis* f.sp. *hordei*, *Bgh*). *Bgh* is an obligate biotroph that obtains its nourishment via haustoria in host epidermal cells; (ii) maize/smut (*U. maydis*, *Um*). As we saw previously, *Um* is a biotroph that colonises the entire leaf tissue via intracellular and intercellular hyphae; and (iii) maize/anthracnose (*Colletotrichum graminicola*, *Cg*). *Cg* is a hemibiotroph that switches from early biotrophic colonisation of epidermal cells to proliferation by necrotrophic hyphae throughout the entire leaf. On the basis of the results obtained from the metabolome and transcriptome analyses, Voll *et al.* (2011) were able to construct models for *Bgh*, *Um* and *Cg* during early biotrophic colonisation (Fig. 9.1). These models revealed similarities between two or more pathosystems: (i) induction of glutamine and asparagine biosynthesis, (ii) reduced activity of the Calvin cycle and/or starch biosynthesis, (iii) increased activity of glycolysis and the tricarboxylic acid (TCA) cycle, and (iv) increased photorespiration and reduced sucrose biosynthesis. Since these changes in primary metabolism occur in three different pathosystems, they are likely to represent part of a common response of cereal primary metabolism during early biotrophic colonisation and highlight the requirement for metabolic energy and the rearrangement of amino acid pools during this phase of the interactions (Voll *et al.*, 2011).

So far, we have examined changes occurring in interactions of plants with biotrophic and hemibiotrophic pathogens. What happens in interactions with necrotrophic pathogens? *Botrytis cinerea* is a necrotrophic fungal pathogen that uses a range of toxins, in addition to the plant's own defences, to kill host cells before feeding (Govrin *et al.*, 2006; Williamson

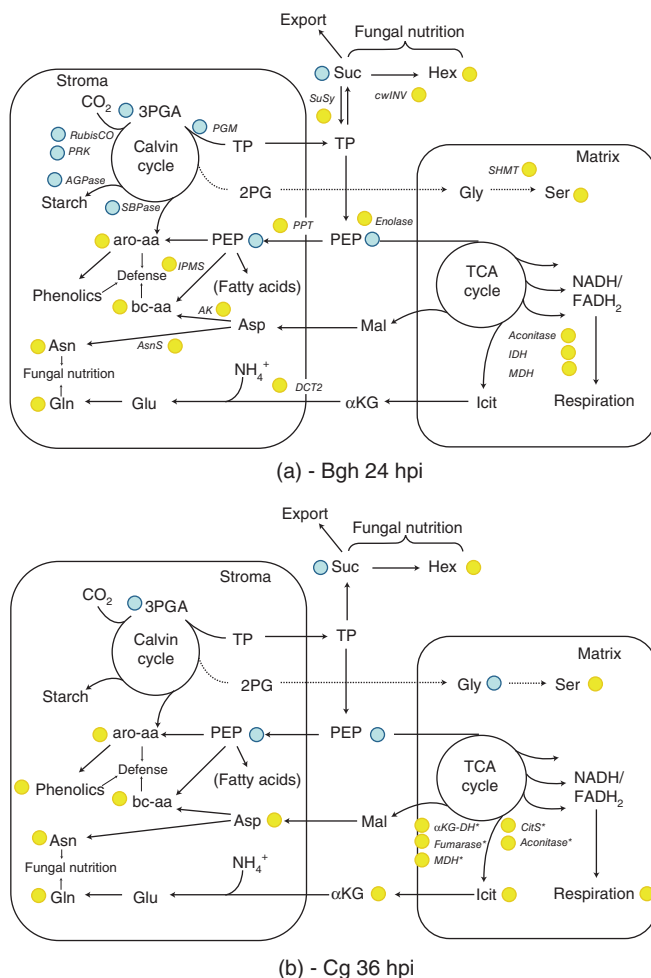


Fig. 9.1 Models of leaf metabolism during early interaction stages. On the basis of the results of combined metabolome and transcriptome analysis, models illustrating the reprogramming of host metabolism during early biotrophic interactions are depicted for *Blumeria graminis* f.sp. *hordei* (Bgh) infected barley leaves at 24hpi (A), *Colletotrichum graminicola* (Cg) infected maize leaves at 36hpi (B) and *Ustilago maydis* (Um) infected maize leaves at 48hpi (C). Please note that for simplicity, C4 metabolism has been omitted from the maize models. Lighter coloured circles – up compared to mock control; darker coloured circles – down compared to mock control. Arrow thickness correlates with the proposed metabolic flux relative to the other depicted metabolic pathways. Amino acids are abbreviated according to three letter code, 2PG, (2-phosphoglycolate); α KG, (α -ketoglutarate) Hex (hexoses); Icit, (isocitrate); PEP, (phosphoenol pyruvate); 3-PGA, (3-phosphoglycerate); Suc, (sucrose); TP (triose phosphates); α KG-DH, (α -ketoglutarate dehydrogenase); AK, (aspartate kinase); AsnS, (asparagine synthetase); CitS, (citrate synthase); cw-INV, (cell wall invertase); DCT2, (dicarboxylate translocator); FBPase2, (fructose-2,6-bisphosphatase); IDH, (isocitrate dehydrogenase); IPMS, (isopropylmalate synthase); MDH, (malate dehydrogenase); PEPC, (PEP carboxylase); PFK2, (phosphofructokinase 2); PFP, (pyrophosphate-dependent phosphofructokinase); PPT, (phosphoenolpyruvate/phosphate translocator); SHMT, (serine hydroxymethyl transferase); SPS, (sucrose phosphate synthase); SuSy, (sucrose synthase). Voll *et al.* 2011. Reproduced with permission from L.M. Voll.

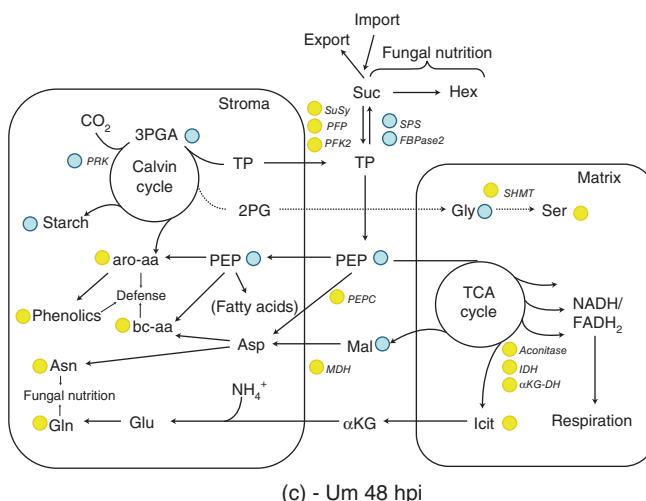


Fig. 9.1 (continued).

et al., 2007). Windram *et al.* (2012) used high resolution transcriptomic analysis to study the chronology and regulation of defence in *Arabidopsis* against *B. cinerea*. They found that approximately one-third of the host genome was differentially expressed during the first 48 hours after infection, with most changes in gene expression occurring before significant lesion development. The data highlight the importance of jasmonic acid/ethylene (JA/ET) in the interaction and also suggest that ethylene (ET) activates auxin biosynthesis during the plant response. The data also suggest a strong repression of ABA signalling during infection of *Arabidopsis* by *B. cinerea*. A particularly striking finding was the down-regulation of photosynthesis and associated processes in response to infection. Down-regulation of chlorophyll biosynthesis started about 14 hours after infection, with reductions in photosynthetic gene expression occurring from about 18 hours after infection (Fig. 9.2). As we have seen previously (Chapter 3 and previous sections), down-regulation of photosynthesis occurs in many plant–pathogen interactions and in compatible interactions might, in part, allow host nitrogen to be reallocated to defence. One of the first responses to infection observed was a dramatic down-regulation of components of the host’s translational machinery, with 74 genes coding for ribosomal proteins down-regulated in three waves at 12, 18 and 28 hours after infection (Fig. 9.2). It was not clear whether this process was mediated by the plant or was an effect of pathogen toxins, but the fact that the changes were early and coordinated suggests a specific function in defence (Windram *et al.*, 2012). Dampening of expression of core components of the circadian clock was also observed, starting at 24 hours after infection. Although this might reflect an attempt by the pathogen to dampen rhythmic defence gene expression, the authors considered it possible that the dampening of circadian clock gene expression might be the result of the reduced levels of translational machinery mentioned previously.

The picture that emerges from these studies is of a complex interplay between pathogen and host, resulting in massive, interconnected changes in host primary and secondary metabolism. These changes reflect a reprogramming of host metabolism, allowing the pathogen to suppress host defences and ensuring the establishment of an appropriate nutritional environment for pathogen growth and reproduction. These studies also highlight an important consideration. It might be assumed that, especially for necrotrophic pathogens, reductions in photosynthesis

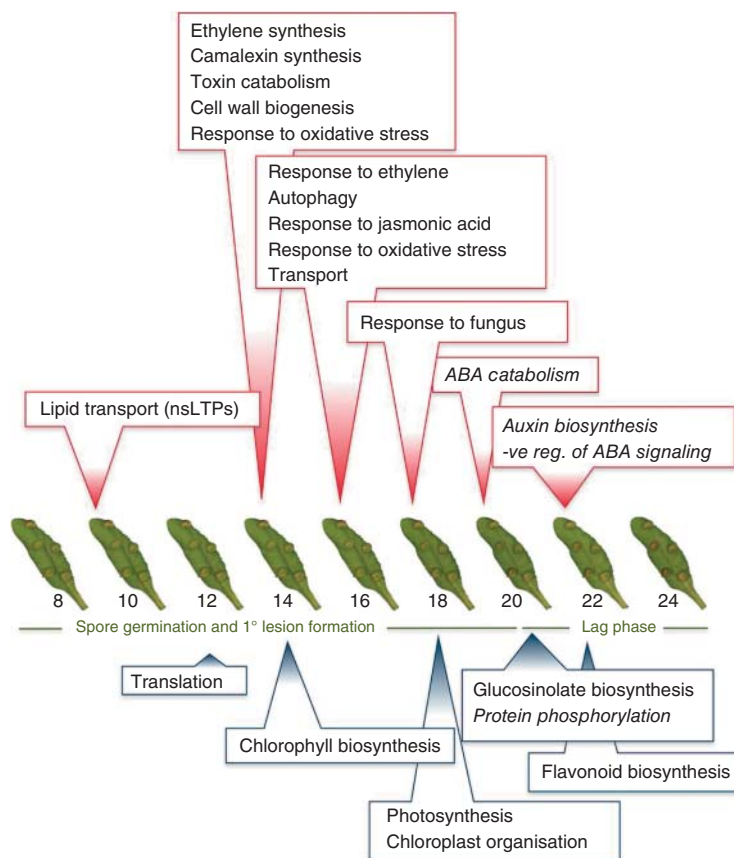


Fig. 9.2 Selected gene ontology (GO) terms overrepresented in clusters of genes differentially expressed after *B. cinerea* infection of *Arabidopsis* leaves. GO terms are aligned with the time of gradient change and/or time of first differential expression of the cluster (in *italics*), with red boxes containing GO terms from up-regulated genes and blue boxes containing GO terms from down-regulated genes. Windram *et al.* 2012. Reproduced with permission of American Society of Plant Biologists.

are likely to be largely attributable to loss of leaf area. As we have seen previously for interactions with *B. cinerea*, and for interactions with biotrophic and hemibiotrophic pathogens, such an assumption would be wrong, as down-regulation of photosynthesis occurs early in these host–pathogen interactions, before symptom development. Photosynthetic metabolism is clearly targeted for down-regulation in infected leaves, but whether such down-regulation is mediated by the host or the pathogen remains uncertain.

9.3 METABOLIC REPROGRAMMING IN INTERACTIONS BETWEEN PLANT AND PARASITIC NEMATODES

Feeding sites of sedentary endoparasitic nematodes are the sole source of nutrients for these parasites, which include *Meloidogyne* spp. and cyst nematodes such as *Heterodera*

schachtii. The latter was found to cause a major reprogramming of primary metabolism in *A. thaliana*, manipulating the plant to redirect nutrients to the nematode-induced sink, especially during the initial phase of giant cell formation (Hofmann *et al.*, 2010). Similarly, the root knot nematode, *Mycosphaerella graminicola*, enhanced nutrient transport towards the induced gall in rice roots, whereas the migratory root rot nematode, *Hirschmanniella oryzae*, induced programmed cell death and oxidative stress and obstructed normal metabolic activity in the root (Kyndt *et al.*, 2012). Interestingly, photosynthesis-related genes were highly up-regulated in galls induced by the root knot nematode (roots are capable of developing chloroplasts under light induction; Flores *et al.*, 1993), whereas root tissues infested with the root rot nematode exhibited a strong suppression of photosynthesis-related gene expression (Kyndt *et al.*, 2012).

9.4 METABOLIC REPROGRAMMING IN PLANT-INSECT INTERACTIONS

As with pathogen infection, insect attack can result in substantial reprogramming of host metabolism, as the attacker tries to deal with host defences and gain access to plant resources. For example, gall-forming insects alter plant growth patterns and modify plant organs in ways that benefit the insect. The gall-forming aphid-like phylloxera parasite, *Daktuloshpairo vitifolia*, was found to induce formation of stomata on the adaxial surface of grape leaves, despite the fact that stomata do not usually form on this surface (Nabity *et al.*, 2013). Induction of stomatal formation occurred close to the site of insect feeding and led to increased assimilation and importation of carbon into the gall. Gene expression associated with the transport of water and nutrients, as well as glycolysis and fermentation, was increased in leaf gall tissues, representing a shift from an autotrophic to a heterotrophic profile. This was associated with a decrease in defence-related gene expression. It appears therefore that induction of stomatal formation by phylloxera reconfigures host leaf metabolism in order to increase carbon gain, perhaps to partially offset the negative impact of gall formation (Nabity *et al.*, 2013).

As we have already seen, plants in a compatible interaction with an attacker need to balance primary metabolism and the need to minimise loss of fitness, with metabolic changes necessary to support defence and minimise the availability of resources to the attacker. For example, in the compatible interaction between tomato and the aphid *Macrosiphum euphorbiae*, transcriptomic and proteomic analysis revealed that up-regulation of genes and proteins associated with defence, was accompanied by down-regulation of gene expression and protein accumulation associated with photosynthesis (Coppola *et al.*, 2013). There was also a down-regulation of genes involved in carbohydrate and water transport, which, together with the down-regulation of photosynthesis, perhaps reflects a strategy by the tomato plant to limit the resources available to the attacking aphids. Interestingly, genes associated with transport of amino acids and nitrogen were up-regulated, suggesting that despite the down-regulation of various aspects of plant primary metabolism, aphids are still able to manipulate plant physiology for their benefit. Coppola *et al.* (2013) suggest that their data indicate a host response that allows reallocation of energy towards defence, while modulating primary metabolism to indirectly reduce performance of the attacking aphids.

9.5 METABOLIC REPROGRAMMING IN INTERACTIONS BETWEEN PLANTS AND PARASITIC ANGIOSPERMS

In the compatible interaction between cowpea and *Striga gesneroides*, the most highly down-regulated genes were those involved in biosynthesis of phenylpropanoids and lignin, biogenesis of primary and secondary cell walls, and SA and JA signal transduction (Huang *et al.*, 2012). Perhaps unsurprisingly, up-regulated genes included those responsible for transport of nitrogen, sulphur and amino acids. This suggests that, in addition to suppressing some host functions to facilitate entry into the host, the parasitic plant also modifies other functions in order to provide a source of nutrition.

9.6 METABOLIC REPROGRAMMING – IS THE PLANT JUST A BYSTANDER IN COMPATIBLE INTERACTIONS?

In an incompatible interaction between a plant and an attacker, the plant clearly has the upper hand – it can activate its defences rapidly, thereby limiting or halting the progress of the attacker. As we have seen, energy and resources are required for these defence responses, and the plant must balance the requirements for defence with those for growth and fitness. As a result, changes in plant growth and reproduction can sometimes be detected in resistant plants in an incompatible interaction. In contrast, in a compatible interaction, the attacker has the upper hand. In this case, the attacker can effect a substantial reprogramming of host metabolism, simultaneously suppressing host defences and modifying host metabolism to ensure an adequate nutrient supply. However, to suggest that the plant is simply a bystander in such interactions would be wrong. The plant can and does respond, and as we have seen previously, some of these host responses can slow down the progress of the attacker, so things do not go entirely smoothly for the parasite or pest. In addition, some plants are able to compensate, at least to some extent, for damage caused by the attacker, thereby ensuring its survival for long enough to produce seed. Importantly, traits that allow plants to tolerate attack could be useful in plant breeding (Bingham & Newton, 2009).

9.7 PLANT RESPONSES TO ATTACK – A LOOK TO THE FUTURE

As the world's population continues to increase, the challenge of feeding the billions of humans spread across the planet becomes more urgent. This challenge will become more difficult as we face the impact of climate change and ever-increasing fuel prices. Protecting crops from the ravages of pathogens, pests and parasitic plants has always been important, and with annual losses from diseases alone accounting for some 15% of total crop production, it will continue to be so. Providing effective protection for crops, thereby reducing losses due to attack, requires an effective armoury. Central to this arsenal is the use of crop varieties that are resistant or tolerant to pathogens, pests and parasitic plants. Understanding how plant primary metabolism responds to attack, how plant metabolism is reprogrammed by attackers and the mechanisms underlying the ability of some plants to tolerate attack is important in the development of crop

varieties with improved resistance or tolerance. In addition, techniques such as chlorophyll fluorescence imaging, which is a useful, non-invasive and non-destructive tool for the study of photosynthetic metabolism, could also be used to provide pre-symptomatic diagnosis of pathogen infection, for example (Rolfe & Scholes, 2010).

Our understanding of how plants respond to biotic challenge has increased enormously in recent years, as advancing technology has allowed us to study plant biotic interactions in increasing detail. As we have seen in this chapter, genomics, metabolomics, proteomics and transcriptomics are being used to study global changes in plant primary and secondary metabolism in response to attack. These studies are providing new insights into the dynamics of the interaction between host and attacker. But the challenge continues, because plant biotic interactions are moulded by the abiotic and biotic environment, as well as by the plant's endogenous circadian clock (Griebel & Zeier, 2008; Kim *et al.*, 2011; Hua, 2013), and these complex interactions await future investigation. Traditionally, studies of the effects of biotic attack on plant primary metabolism and on plant physiology have lagged behind those of plant defensive responses. But this situation is changing and rightly so, because plant defence does not occur in isolation from other aspects of plant metabolism. These are exciting times to be studying plant biotic interactions. The more we discover about plant interactions with the environment from which they cannot escape, the more we will realise just how remarkable plants are!

RECOMMENDED READING

- Baldwin IT, 2012. Training a new generation of biologists: the genome-enabled field biologists. *Proceedings of the American Philosophical Society* **156**, 205–214.
- Brown JKM, Rant JC, 2013. Fitness costs and trade-offs of disease resistance and their consequences for breeding arable crops. *Plant Pathology* **62**(Supplement 1), 83–95.
- Evans LT, 2001. *Feeding the ten billion. Plants and population growth*. Cambridge: Cambridge University Press.
- Hodson MJ, Bryant JA, 2012. *Functional biology of plants*. Oxford: Wiley-Blackwell.

REFERENCES

- Bingham IJ, Newton AC, 2009. Crop tolerance of foliar pathogens: possible mechanisms and potential for exploitation. In: Walters DR, ed. *Disease control in crops: biological and environmentally friendly approaches*. Oxford: Wiley-Blackwell, 142–161.
- Coppola V, Coppola M, Rocco M, Diglio MC, D'Ambrosio C, Renzone G, Martinelli R, Scaloni A, Pennacchio F, Rao R, Corrado G, 2013. Transcriptomic and proteomic analysis of a compatible tomato-aphid interaction reveals a predominant salicylic acid-dependent plant response. *BMC Genomics* **14**, 515.
- Doehlemann G, Wahl R, Horst RJ, Voll LM, Poree F, Stitt M, Pons-Kuhnemann J, Sonnewald U, Kahmann R, Kämper J, 2008. Reprogramming a maize plant: transcriptional and metabolic changes induced by the fungal biotroph *Ustilago maydis*. *The Plant Journal* **56**, 181–195.
- Flores HE, Dai YR, Cuello JL, Maldonadomendoza IE, Loyolavargas VM, 1993. Green roots – photosynthesis and photoautotrophy in an underground plant organ. *Plant Physiology* **101**, 363–371.
- Glazebrook J, 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**, 205–227.
- Govrin EM, Rachmilevitch S, Tiwari BS, Solomon M, Levine A, 2006. An elicitor from *Botrytis cinerea* induces the hypersensitive response in *Arabidopsis thaliana* and other plants and promotes the gray mold disease. *Phytopathology* **96**, 299–307.

- Griebel T, Zeier J, 2008. Light regulation and daytime dependency of inducible plant defences in Arabidopsis: phytochrome signalling controls systemic acquired resistance rather than local defense. *Plant Physiology* **147**, 790–801.
- Hofmann J, El Ashry AEN, Anwar S, Erban A, Kopka J, Grundler F, 2010. Metabolic profiling reveals local and systemic responses of host plants to nematode parasitism. *The Plant Journal* **62**, 1058–1071.
- Horst RJ, Engelsdorf T, Sonnewald U, Voll LM, 2008. Infection of maize leaves with *Ustilago maydis* prevents establishment of C-4 photosynthesis. *Journal of Plant Physiology* **165**, 19–28.
- Hua J, 2013. Modulation of plant immunity by light, circadian rhythm, and temperature. *Current Opinion in Plant Biology* **16**, 406–413.
- Huang K, Mellor KE, Paul SN, Lawson MJ, Mackey AJ, Timko MP, 2012. Global changes in gene expression during compatible and incompatible interactions of cowpea (*Vigna unguiculata* L.) with the root parasitic angiosperm *Striga gesnerioides*. *BMC Genomics* **13**, 402.
- Kim S-G, Yon F, Gaquerel E, Gulati J, Baldwin IT, 2011. Tissue specific diurnal rhythms of metabolites and their regulation during herbivore attack in a native tobacco, *Nicotiana attenuata*. *PLoS One* **6**(10), e26214.
- Kyndt T, Denil S, Haegeman A, Trooskens G, Bauters L, Van Crielinge WV, De Meyer T, Gheysen G, 2012. Transcriptional reprogramming by root knot and migratory nematode infection in rice. *New Phytologist* **196**, 887–900.
- Nabity PD, Haus MJ, Berenbaum MR, DeLucia EH, 2013. Leaf-galling phylloxera on grapes reprograms host metabolism and morphology. *Proceedings of the National Academy of Sciences USA* **110**, 16663–16668.
- Parker D, Beckman M, Zubair H, Enot DP, Caracuel-Rios Z, Overly DP, Snowdon S, Talbot NJ, Draper J, 2009. Metabolomic analysis reveals a common pattern of metabolic re-programming during invasion of three host plant species by *Magnaporthe grisea*. *The Plant Journal* **59**, 723–737.
- Rolfe SA, Scholes JD, 2010. Chlorophyll fluorescence imaging of plant-pathogen interactions. *Protoplasma* **247**, 163–175.
- Talbot NJ, 2003. On the trail of a cereal killer: exploring the biology of *Magnaporthe grisea*. *Annual Review of Microbiology* **57**, 177–202.
- Voll LM, Horst RJ, Voitsik A-M, Zajic D, Samans B, Pons-Kuhnemann J, Doehlemann G, Munch S, Wahl R, Molitor A, Hofmann J, Schmeidl A, Waller F, Deising HB, Kahmann R, Kämper J, Kogel K-H, Sonnewald U, 2011. Common motifs in the response of cereal primary metabolism to fungal pathogens are not based on similar transcriptional reprogramming. *Frontiers in Plant Science* **2**, 39. doi: 10.3389/fpls.2011.00039.
- Wang D, Pajeroska-Mukhtar K, Culler AH, Dong X, 2007. Salicylic acid inhibits pathogen growth in plants through repression of the auxin signalling pathway. *Current Biology* **17**, 1784–1790.
- Ward JL, Forcat S, Beckmann M, Bennett M, Miller SJ, Baker JM, Hawkins ND, Vermeer CP, Lu C, Lin W, Truman WM, Beale MH, Draper J, Mansfield JW, Grant M, 2010. The metabolic transition during disease following infection of *Arabidopsis thaliana* by *Pseudomonas syringae* pv. *tomato*. *The Plant Journal* **63**, 443–457.
- Williamson B, Tudzynski B, Tudzynski P, van Kan JAL, 2007. *Botrytis cinerea*: the cause of grey mould disease. *Molecular Plant Pathology* **8**, 561–580.
- Windram O, Madhou P, McHattie S, Hill C, Hickman R, Cooke E, Jenkins DJ, Penfold CA, Baxter L, Breeze E, Kiddle SJ, Rhodes J, Atwell S, Kliebenstein DJ, Kim Y-S, Stegle O, Borgwardt K, Zhang C, Tabrett A, Legaie R, Moore J, Finkensadt B, Wild DL, Mead A, Rand D, Benyon J, Ott S, Buchanan-Wollaston V, Denby KJ, 2012. *Arabidopsis* defense against *Botrytis cinerea*: chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. *The Plant Cell* **24**, 3530–3557.

Index

- Abscisic acid (ABA) 73, 132–4, 137, 146, 181
 And insect attack 197
 In plants attacked by parasitic plants 200, 202
 And plant defence 182, 184
 And vascular wilts 181–2
- Agapeta zoegana* 166
- Agelastica alni* 139
- Albugo candida* 41, 42, 45, 115–16
- Alternaria brassicicola* 185–6
- Alternaria solani* 114
- Alternative oxidase (AOX) 88, 91, 93–5, 100
- Amino acid transporter protein (AAT2p) 8–9
- Ammonium uptake 152
- Aphis fabae* 141
- Aphis glycines* 67
- Aquaporin genes 164
- Arabidopsis thaliana* 41, 50, 64–5, 93, 100, 164, 181
- Armillaria mellea* 2
- Asparagine synthetase (AS) 158, 161
- Assimilate partitioning
 In plants infected by pathogens 113
 Mechanisms, in pathogen-infected plants 114
- Attacker, different types 1
 Bacteria 3
 Fungi 2
 Insects 12
 Microorganisms 2
 Nematodes 11
 Parasitic plants 16
 Phytoplasmas 3
 Viruses 3
- Auxin 185
 And clubroot 188
 And defence against insect herbivores 198
 And gall formation following insect attack 198
 And nematodes 189
 In plants attacked by parasitic plants 202
 And plant defence 185
- Barley yellow dwarf virus* (BYDV) 26
- Biotrophs 2, 4, 6, 114
- Blumeria graminis* f.sp. *hordei* 130, 132, 216
- Botrytis cinerea* 163, 183, 186, 216, 219
- Brassinosteroids 189
- Brown rust, barley 90–91, 115
- Cab2* gene 59
- Cabbage white butterfly 13
- Carbohydrate partitioning and metabolism
 In interactions between plants and parasitic angiosperms 123
 In plant-pathogen interactions 113
 In plant-insect herbivore interactions 121
- Cassitha pubescens* 75
- Chlorophyll content
 In diseased plants 42
- Chlorophyll fluorescence 46, 57, 72, 74
- Circadian clock, and plant immunity 195
- Coleoptera 12
- Colletotrichum higginsianum* 6
- Colletotrichum lindemuthianum* 51, 130, 161
- Colonisation of host tissues 9
- Compensatory regrowth
 Extrinsic mechanisms 34
 Intrinsic mechanisms 34
- Coronatine 134
- Crop growth
 Effects of infection and infestation 24
- Cucumber mosaic virus* (CMV) 3, 96, 114
- Cuscuta* 16, 19
- Cuscuta campestris* 78–9, 146, 147
- Cuscuta reflexa* 20, 78, 124, 169

- Cytokinins 120, 137, 190, 192
 And defence against insect herbivory 199
 And green islands 189
 And leaf-mining and gall-forming insects 200–202
 And pathogen-induced gall formation 191
 And plant defence 191–2
 In plants attacked by parasitic plants 203
- Danaus plexipus* 168
 Deer, black-tailed 32
 DELLA proteins 194
Dendroctonus ponderosae 66
Diabrotica virgifera virgifera 197
 Dihydrozeatin riboside 190
Diprion piri 70
 Dodder 16, 19
- Empoasca fabae* 141
Erysiphe graminis 89, 90
Erysiphe graminis f.sp. *avenae* 42
Erysiphe pisi 42
 Ethylene 181,
 Extrahaustorial matrix 5, 7, 9
 Extrahaustorial membrane 5, 7–8
- Fusaric acid 137
Fusarium culmorum 159
Fusarium oxysporum f.sp. *ciceris* 29
Fusarium oxysporum f.sp. *cubense* 136–7
 Fusicoccin 134
- GABA shunt 87, 91, 163
Gaeumannomyces graminis 2, 159, 160
 Gibberellins 194, 196, 204
Globodera pallida 61, 139
 Glutamate metabolism and plant disease 162
 Glutamine:2-oxoglutarate aminotransferase (GOGAT) 152, 154, 157, 162–3
 Glutamine synthetase (GS) 152, 154, 157, 161–3
 Glycolysis 87–8, 99, 216
Gnorimoschema gallaesolidaginis 198, 199
 Green islands
 In leaves attacked by insects 200
 In leaves infected with fungal pathogens 46, 47
- Haustorium
 of pathogenic fungi 4, 6–9
 of parasitic plants 16–19, 169
Helicoverpa zea 140
- Heliothis virescens* 197–8
Helminthosporium victoriae 92
 Hemibiotrophs 2, 4–6, 114
 Herbivory
 Compensation 33–6
 Tolerance of 33–6
 Herbivore-associated molecular patterns (HAMPs) 16
Heterodera avenae 139
Heterodera glycines 61
Heterodera schachtii 164
Heterodera trifolii 168–9
 Hexose transporter (*HXT1*) 7–9
 Hormonal changes
 And insect attack 197
 In attacked plants 180
 In plants attacked by parasitic plants 200
 In plants responding to pathogens 180
 Host-pathogen interface 4–5
Hyaloperonospora parasitica 6
 Hydraulic conductivity 138
 Hypersensitive response (HR) 132, 215
- Insect
 Feeding 13–16
 Oral secretions (OS) 16
 Insects 12
 Chewing 31
 Generalists 13
 Haustellate 13
 Heteroptera 14
 Mandibulate 13
 Monophagous 12–13
 Mouthparts 13–16
 Oligophagous 12–13
 Polyphagous 13
 Sap-sucking 31
 Specialists 13
 Intracellular hyphae 5–6, 9
 Invertase 7, 9, 115–18
 Apoplastic 115, 118–19
 Cell wall-associated (*CWINV2*) 8
 Cell wall-bound 115, 117
 Soluble 115
- Jasmonic acid 121, 181, 184, 197–9, 204, 215
- Lepidoptera 12
Leptinotarsa decimlineata 13, 66
Lymantria dispar 122

- Magnaporthe grisea* 161, 192, 215
Manduca sexta 66, 121, 181
 Mannitol 10, 115
 Mannitol dehydrogenase 115
Marssonina brunnea 51
Meloidogyne ethiopica 61–2, 138
Meloidogyne incognita 11, 13, 61, 138
Meloidogyne javanica 138
 Metabolic reprogramming
 In interactions between plants and parasitic nematodes 219
 In interactions between plants and parasitic plants 221
 In plant-insect interactions 220
 In plant-pathogen interactions 214
 Methyl jasmonate 167
Microsphaera alphitoides 41, 105–6
 Mineral nutrition
 Effects of foliar pathogens 155
 Effects of parasitic angiosperms 169
 Effects of root-infecting pathogens 159
 In plant-insect interactions 164
 In plant-nematode interactions 163
 In plant-pathogen interactions 155
 Mistletoes 146
 Moose 33
 Movement proteins 120
Murgantia histrionica 67–8
Mycosphaerella graminicola 51
Mycosphaerella pinodes 28
Myzus persicae 13

 Necrotrophs 2, 114
 Nematodes 11
 Cyst 12
 Root-knot 12
 Stylet 12
Nezara viridula 67, 69
 Nitrate reductase (NR) 154, 172
 Nitrite reductase (NiR) 154
 Nitrate uptake 152–3, 157
 Nitric oxide (NO) 100
 Nitrogen allocation
 Following herbivory 168
 Following methyl jasmonate treatment 167
 Nitrogen assimilation 154
 Nitrogen metabolism
 And plant defence against pathogens 161
 Effects of parasitic plants 169–70, 172
 In mildewed barley 157–8
 Nitrogen uptake and root herbivory 165–6

 Non-cyclic photophosphorylation
 In diseased plants 42–3

Ola x phyllanthi 20, 124, 172–3
Olpidium brassicae 4
 OPDA (12-oxo-phytodienoic acid) 59
Ophiostoma ulmi 135
 Oral secretions (OS) 197
Orobanche 16
Orobanche aegyptiaca 36–7
Orobanche cernua 75, 123
Orobanche crenata 20
 Orthoptera 12
 Oxidative pentose phosphate pathway 87–8, 96, 99

 Parasites 2
 Parasitic plants 16
 Haustorium 16–19
 Facultative 16
 Hemiparasitic 16
 Holoparasitic 16
 Obligate 16
 Pathogens 2
 Phosphate uptake 154–5
 Photoinhibition 104–5
 Photorespiration 87–9
 In attacked plants 104–5
 Photosynthesis
 Down-regulation following pathogen attack 218–19
 Following oviposition 70
 Following root infection by fungal pathogens 52
 In attacked plants 40–86
 In diseased plants 40–60
 In incompatible plant-fungal interactions 54
 In localised regions of infected leaves 45
 In plants attacked by chewing insects 64
 In plants attacked by piercing-sucking insects 67
 In plants infected with bacterial pathogens 57
 In plants infected with biotrophic fungal pathogens 41–50
 In plants infected with hemibiotrophic and necrotrophic fungal pathogens 51
 In plants infected with hemiparasites 72
 In plants infected with holoparasites 75
 In plants infected with nematodes 60
 In plants infected with parasitic plants 72

- Photosynthesis (*continued*)
 In plants infected with the clubroot pathogen 49
 In plants infected with viruses 59
 In plants infested with insects 64–72
 In uninfected leaves of otherwise infected plants 48
 Or defence 70
- Photosystem I (PSI) 44, 46, 57
 Photosystem II (PSII) 44, 46, 57, 59, 60, 67, 75–6
- Phyllonorycter blancardella* 141, 142, 144–5, 200
- Phytophthora cinnamomi* 136
Phytophthora infestans 2, 51, 131
Phytophthora nicotianiae 54, 91, 119
Phytophthora ramorum 52
Pieris rapae 181
Pieris brassicae 13, 72
- Plant growth
 Effects of biotrophic pathogens 25
 Effects of infection and infestation 24
 Effects of insect herbivores 30
 Effects of necrotrophic pathogens 27
 Effects of nematodes 29
 Effects of parasitic plants 36
 Effects of pathogens 24–9
 Effects of vascular wilts 28
 Effects of vertebrate herbivores 31
- Plasmodiophora brassicae* 4, 49, 50, 188
Plasmopara viticola 132
- Polyamines 193
Popilla japonica 139
- Potassium uptake 154–6
 Potato cyst nematode 61
Potato virus Y (PVY) 60
- Powdery mildew
 of barley 25, 28, 44, 49
- Powdery mildews 3–4
PR-1 117–18
Pratylenchus coffeae 63
Pratylenchus neglectus 30
Pratylenchus penetrans 63–4
Pseudomonas solanacearum 135
Pseudomonas syringae 2, 57, 59, 161
P. syringae pv. *tabaci*, 192
P. syringae pv. *tomato*, 93–5
P. syringae pv. *tomato* DC3000 57, 134, 183, 216
P. syringae pv. *tagetis*, 59
Pseudoperonospora cubensis 107
- Puccinia allii* 47
Puccinia hordei 8, 48
Puccinia striiformis 114
Puccinia triticina 91
 Putrescine 193
Pyrenopeziza brassicae 190
Pyrenophora teres 27, 89–90
Pythium aphanidermatum 52
- Ralstonia solanacearum* 28
RbcS gene 59
 Reactive oxygen species (ROS) 88, 100, 115, 162, 215
- Respiration 87
 Effects of bacterial pathogens 93
 Effects of fungal and oomycete pathogens 89
 Effects of insect herbivores 102
 Effects of parasitic plants 103
 Effects of viruses 95
- Rhinanthus minor* 16, 18, 75, 172, 202
Rhinanthus serotinus 145
Rhynchosporium secalis (*commune*) 28, 131, 156
Rice black-streaked dwarf virus (RBSDV) 26
 Ribulose-1, 5-bisphosphate carboxylase (Rubisco)
 In diseased plants 44–5, 59, 118
- Root knot nematode 61
- Rusts 3, 4
- Salicylic acid 100, 181, 184, 204, 215
 Saprotrophs 2
Senecio vulgaris 25
Septoria nodorum 114
Septoria tritici blotch (STB) 51
 Slash and burn strategy 161–2
 Snails 32
 Solute accumulation, at sites of fungal infection 155
 Spermidine 193
 Spermine 193
Spodoptera littoralis 197
 Stomatal behaviour
 And plant immunity 133
 In diseased plants 42, 130
 Stomatal conductance 130, 132, 137, 138, 142, 144
Striga asiatica 72
Striga gesnerioides 36
Striga hermonthica 16, 36, 72, 145, 146, 203
 Sucrose transporter protein (Srt1) 10

- Sugar uptake
by pathogenic fungi 10
Sulphur assimilation 158–9
Sulphate uptake 154
Symptoms, caused by pathogens, herbivores and
parasitic plants 20, 21
Syncytium 12, 164
- Tetraopes tetraophthalmus* 168
Thysanoptera 12
Tobacco Mosaic Virus (TMV) 3, 95, 96, 100
Tolerance
of herbivory 33–6
Transpiration 131, 133, 134, 136, 137, 138, 139,
140, 141, 142, 144
Tricarboxylic acid (TCA) cycle 87, 88, 91, 99,
163, 216
Trichoplusia ni 64, 65, 102
Tyloses 28, 29, 135
- Uf-INVI* (invertase) 7
Uromyces appendiculatus 41, 114, 130
Uromyces fabae 7, 115
Uromyces muscari 46, 47
Uromyces phaseoli 155
Uromyces vignae 5
Ustilago maydis 10, 162, 214
- Verticillium dahliae* 28, 54, 136
Verticillium albo-atrum 29, 181, 182
- Victorin (HV-toxin) 92, 93
Viroids 3
Virtual lesion 51
Virus classification 4
Viscum album 146
- Water relations
Effects of foliar pathogens 129
Effects of insect herbivores 139
Effects of nematodes 138
Effects of parasitic angiosperms 144
Effects of root rot pathogens 136
Effects of vascular wilt pathogens 135
Water uptake and transport
Effects of foliar pathogens 134
Water use efficiency (WUE) 142
Wolbachia 200
Yellow rust, of wheat 25
- Xanthomonas campestris* pv. *campestris* 134
Xanthomonas campestris pv. *vesicatoria* 162
Xanthomonas citri pv. *citri* (Xcc) 59
Xanthomonas oryzae pv. *oryzae* 196
Xanthomonas vesicatoria 93
Xylella fastidiosa 136
- Zeatin riboside 190

WILEY END USER LICENSE AGREEMENT

Go to www.wiley.com/go/eula to access Wiley's ebook EULA.